ANNUAL REPORT OF PROGRAM ACTIVITIES NATIONAL INSTITUTE ON AGING

Fiscal Year 1975

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health

ANNUAL REPORT

NATIONAL INSTITUTE ON AGING

REPORT OF PROGRAM ACTIVITIES

July 1, 1974 - June 30, 1975

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OFFICE OF THE DIRECTOR

Establishment of the National Institute on Aging

On May 31, 1974 the President signed the Research on Aging Act of 1974 (Public Law 93-296) directing the Secretary to establish a National Institute on Aging, and on October 7, 1974 that Institute was legally established by a formal announcement in the Federal Register.

Because of the very broad requirements of the Act, an Interagency Committee was formed to assist NIH in its implementation. The implementation plan, concerned both with making the Institute a functional entity and with other requirements of the Act, was submitted to the Assistant Secretary for Health by the Acting Director of NIH on January 8, 1975. NIH is now carrying out those parts of the plan concerned with the new Institute.

The Mission of the Institute

The motivation of Congress in establishing the Institute was expressed in the following words.

> "The Congress finds and declares that--
>
> (1) the study of the aging process, the one biological condition common to all, has not received research support commensurate with its effects on the lives of every individual;
>
> (2) in addition to the physical infirmities resulting from advanced age, the economic, social, and psychological factors associated with aging operate to exclude millions of older Americans from the full life and the place in our society to which their years of service and experience entitle them;
>
> (3) recent research efforts point the way toward alleviation of the problems of old age by extending the healthy middle years of life;
>
> (4) there is no American institution that has undertaken comprehensive systematic and intensive studies of the biomedical and behavioral aspects of aging and the related training of necessary personnel;
>
> (5) the establishment of a National Institute on Aging within the National Institutes of Health will meet the need for such an institution."

Congress stated the mission of the National Institute on Aging as follows.

> "The Secretary shall establish in the Service an institute to be known as the National Institute on Aging for the conduct and

support of biomedical, social, and behavioral research and training related to the aging process and diseases and other special problems and needs of the aged."

The Transfer of NIH Programs on Aging to NIA

NIH's program on aging has been localized in the National Institute of Child Health and Human Development in recent years. Intramural research has been conducted by the Gerontology Research Center, NICHD and extramural research supported by the Adult Development and Aging Branch, NICHD. NICHD's staff, facilities, and extramural projects concerned with aging will be transferred to the National Institute on Aging to form the nucleus of its research and training program. Since NIH's programs on aging were so sharply localized within NICHD, no transfer of extramural and intramural components from other NIH Institutes is necessary at this time. Because of NIH's prior support of aging research and training, the new Institute will come into being with a small but diversified program of aging research.

The National Advisory Council on Aging

The Research on Aging Act of 1974 called for the creation of a National Advisory Council on Aging to advise the Secretary on the programs relating to aging that are administered by him and on matters that relate to the Institute. That Council has been chartered, and its initial members have been appointed. The Council held its first meeting in May and its second in June.

Staffing

A Search Committee composed of representatives from NIH is assisting the Director of NIH in the selection of a Director for NIA.

The current plans for staffing include the transfer to the Institute of the 155 positions of the Gerontology Research Center, the 10 positions of the Adult Development and Aging Branch, and of 8 other positions from NICHD. The Office of the Director of NIH has been given authority for 5 other positions. Two of the latter have been filled by hiring a Program Planning Officer and an Administrative Officer. Selection of an Executive Officer is in progress. The total number of positions listed above for the Office of the Director and for the Extramural Program is 23.

Budget

The budgets of NICHD for aging research for fiscal years 1974 and 1975 and the President's budget for NIA for fiscal year 1976 are tabulated below.

Aging Research and Training Budgets
(In thousands of dollars)

Fiscal Year	1974	1975	1976
Research Grants	$8,601	$7,592	$7,937
Research Career Development Awards	31	75	75

Fiscal Year	1974	1975	1976
Evaluation	0	23	23
	$8,632	$7,690	$8,035
Training Grants	$1,519	$1,156	$1,133
Fellowships	306	661	629
	$1,825	$1,817	$1,762
Contracts	665	800	702
Intramural Research	$4,690	$4,548	$4,741
Research Management and Program Services	732	867	950
	$16,544	$15,722	$16,190

Plan for a Research Program on Aging

The National Research on Aging Act of 1974 contained the following statement.

> "Sec. 464. (a) The Secretary, in consultation with the Institute and the National Advisory Council on Aging and such other appropriate advisory bodies as he may establish, shall within one year after the effective date of this section develop a plan for a research program on aging designed to coordinate and promote research into the biological, medical, psychological, social, educational, and economic aspects of aging. Such program shall be carried out, as to research involving the functions of the Institute, primarily through the Institute, and as to other research shall be carried out through any other institute established by or under other provisions of this Act or through any appropriate agency or other organizational unit within the Department of Health, Education, and Welfare.
>
> "(b) Upon its completion, the plan for a research program on aging, required by subsection (a) of this section, shall be transmitted to the Congress and to the President and shall set forth the staffing and funding requirements to carry out such program."

Work on the required plan is proceeding. Institute staff and the National Advisory Council on Aging as specified in the Act are involved in the planning. An Interagency Advisory Committee for Research on Aging has been established to help formulate the plan. Panels of consultants concerned with specific aspects of aging will probably be created. A document of intent with regard to the scope of the plan was submitted to Congress on May 31, 1975. A detailed plan is now in the process of being created.

Public Information

The Menopause, a booklet for the public based on a previous publication for the scientific community, was published for the Adult Development and Aging Branch by the G.P.O.

A book entitled "Epidemiology of Aging," edited by Drs. Adrian Ostfeld and Don Gibson, giving a summary report and selected papers from a research conference on the epidemiology of aging was also published for the Branch by the G.P.O.

Each year for some years, the Adult Development and Aging Branch has published a document entitled The Extramural Program of Research on Aging of the National Institute of Child Health and Human Development describing in detail its program. The transfer of programs to NIA has slowed the publication of this document, but it will be resumed.

Research

A little more than $13 million was spent on intramural and extramural research in fiscal year 1975.

INTRAMURAL RESEARCH

The program of the Gerontology Research Center is organized under four Laboratories and Branches, the Laboratory of Behavioral Sciences, the Clinical Physiology Branch, the Laboratory of Cellular and Comparative Physiology, and the Laboratory of Molecular Aging and is run by staff in the Center's 154 budgeted positions. The Office of the Chief of the Center provides research services and supervises the Collaborative Guest Scientist Program.

Research services include the development of instrumentation, electronic data processing, photography and arts, animal services, and library services. Housing and care was provided for 10,000 rats and 13,000 mice in addition to smaller numbers of guinea pigs, rabbits, hamsters, beagles, and monkeys. The library staff continued to search out and index the world literature relating to all aspects of aging. Twenty-nine visiting scientists with 34 supporting personnel worked at the Center.

Studies of intellectual performance continued. Great care must be taken in the analysis of these studies because of the many factors determining that performance. Cohort effects arise because persons born during a particular period will have unique experiences attributable to the continually changing characteristics of our culture. However, whether age comparisons are made longitudinally, cross-sectionally, or within cohorts the results are similar--learning and memory decline with age and the decline is most rapid in older persons. Problem-solving ability was found to decline with age. However, well-practiced subjects (those who have had the opportunity to solve hundreds of problems) attained a level of performance similar to that of young adults. Some well-practiced persons in their 90's were able to solve difficult problems well.

The longitudinal study of aging involving some 600 men continued to generate important information. Analysis showed a difference in the mortality experience of subjects who did or did not exercise regularly. The sedentary group died 2½ years earlier than the active group. This earlier mortality was largely due to an increase in vascular disease.

Other Biological Aging ($1,704,116)

Adelman (PP-Adelman):* Age-Dependent Control of Enzyme Adaptation
Adelman: Effects of Senescence on Enzyme Regulation
Bierman (PP-Bierman): The Effect of Aging on the Proliferative Response of Primate Aortic Smooth Muscle Cells in Tissue Culture to Lipoproteins, Insulin, and Estrogens
Bierman (PP-Bierman): Study of the Interrelation among Aging, Diabetes Mellitus and Atherosclerosis in Man with Use of Aortic Smooth Muscle Cells in Culture
Black (PP-deDuve): Subcellular Events in Arterial Disease
Bornstein (PP-Bierman): The Role of Errors in Protein Synthesis in the Aging Process
Brown (PP-Adelman): A Biochemical Test for the Feasibility of Genetic Error Theory of Aging
Britton: Regulation of Insulin Structure during Aging
Busse (PP-Maddox): The First and Second Longitudinal Studies of Aging
Dill: Cardiovascular Adaptation to Desert and Mountain
Finch: Gene Function during Postnatal Development and Aging
Fink: Protein Metabolism in Aging Mosquitoes
Glomset (PP-Bierman): The Effect of Aging on Plasma Lipoprotein Cholestrerol Transport
Goldthwaite: Regulation of Leaf Senescence by Plant Hormones
Griminger: Effects of Age and Trauma on Nutrient Requirements
Hirsch & McIntosh (PP-Ham): Studies on Aging with the Small Nematode, caenorhabditis elegans
Holloszy: Exercise Induced Biochemical and Anatomic Adaptations
Hinck: A Multivariate Analysis of Mortality Differentials
King: Bioenergetics of Aging: Facts and Mechanisms
King: Bioenergetics of Aging: Facts and Mechanisms
Lang: Metals and Nucleic Acids in Growth and Aging
Lowenstein:* Lipogenesis as a Function of Aging
Lowenstein: Aging in the Kidney
Martin:* Aging as a Consequence of in utero treatment
Masoro: An Approach to the Biochemical Basis of Aging
Mill: The Role of Free Radicals and Antioxidants in Aging
Ohno: Sex and Aging in Mutant Mice
Peters (PP-Sinex): Aging Changes in the Anatomy of the Rat Auditory System
Pittendrigh: Circadian Organization and the Aging Process
Porta: Dietary Factors in Aging
Privett: Relationships of Diet and air pollutants to aging
Ross (PP-Bierman): Effects of Age on the Structure and Function of the Arterial Endothelium in Normal Primates and in Experimental Atherosclerosis
Russell (PP-Russell): Mechanisms of Life-Shortening Effects of W-locus Alleles
Scrimshaw:* Protein Needs of Elderly People
Templeton: Age Change in Left Ventricular Contractile Function
Vaughan: Aging and Protein Cross-Linkage
Whissel-Buechy (PP-Eichorn): Investigation of the Genetic Basis of Continuous Variation
Willis: Analysis of Development in Insects
Woodbury (PP-Maddox): Data Analysis in Longitudinal Studies
Young: Aging and Metabolism in Skeletal Muscle

Neurologic Aging ($410,331)

Bondareff: Age Changes in the Neuronal Microenvironment
Fonda: GABA Metabolism - A Biochemical Key to Mental Aging
Harkins: Age Changes in Averaged Cortical Evoked Potentials
Hinds (PP-Sinex): Aging in the Olfactory Bulb
McNary (PP-Sinex): Study of Aging on the Morphology and Vasculature of the Auditory System with Specific Reference to the Cochlea
Obrist (PP-Maddox): Cerebral Blood Flow and Vascular Reactivity in Senescence
Riegle: The Effect of Aging on Hypothalamic Control Systems
Schaie: Age Difference in CNS, ANS and Sensory-Motor Function
Schiffman: Gustatory and Olfactory Quality Changes with Age
Stoffolano: Effect of Aging on Chemoreceptor Sensillia

Cognitive Change with Age ($684,019)

Campbell: Animal Models of Declining Memory in the Aged
Davis:* Aging in Monkeys
Eisdorfer: Learning and Psychophysiological Studies in the Aged
Elias: Age Effects on Verbal and Non-Verbal Processes
Fozard:* Mental Performance and Aging
Kinsbourne (PP-Maddox): Age Effects on Performance and its Cerebral Lateralization
Marsh (PP-Maddox): Cognitive and Physiological Changes with Aging
McGaugh:* Psychophysiology of Memory and Aging
Mistler-Lachman: Changes in Semantic Memory in Maturity and Old Age
Schaie: Cognitive Behavior in Maturity and Old Age
Smith: Interaction between Human Aging and Memory
Thompson: Information Processing and Aging
Vanderplas:* Incremental Cognitive Functions in Aging

Other Psychologic Aging ($256,728)

Anderson: Aging and the Long-Term Biobehavioral Effects of Stress
Crockett: Personal Constructs, Aging, and Impression Formation
Hahn (PP-Eichorn): Ego Functioning, Coping and Defense
Kelly: Aging, Hypertension, and Behavior
Ostfeld: Relocation in Old Age: Health and Psychological Impact
Peskin (PP-Eichorn): Stage Developmental Antecedents of Psychological Health
Sprott (PP-Russell):** Age-Dependent Behavioral Changes in Mice

Societal Aspects of Aging ($674,101)

Clark (PP-Lowenthal): Ethnic Identity and Adult Development
Eichorn (PP-Eichorn): Levels and Patterns of Mental and Physical Development among Kinships
Higgins: Study of Human Aging in a Defined Community
Havighurst: High Level Productivity after Age Seventy
Kalish: Social Gerontology of Religion and the Clergy
Langer (PP-Eichorn): Study of Operational and Moral Reasoning in Families
Lowenthal (PP-Lowenthal): Longitudinal Study of Transitions
Myers: Study of Models for Forecasting Future U.S. Population
Ruffini: Social Gerontology

Skolinich (PP-Eichorn): Participation in Social Networks
Silverman: A Cross-Cultural Study on the Treatment of the Aged
Todd: Social Gerontology

TRAINING

Psychology and Social Sciences ($866,536)

Botwinick: Psychology - Geriatrics
Loeb: Graduate Research Training Program in Aging
Lowenthal: Adult Development and Aging
Meyer: Developmental Psychology - Development and Aging
Peterson: Interuniversity Training - Adult Development and Aging
Rosenberg: Training in Social Gerontology
Taylor: Interdisciplinary Training in Gerontology

Biologic Sciences ($389,445)

Horvath: Physiology of Exercise and Stress
Huang: Age-Dependent Nucleo-Cytoplasmic Interaction
Kohn: Aging of Mammalian Tissues
Rockstein: Gerontology
Sanadi: Biochemical and Biophysical Basis of Aging
Sinex: Biochemistry of Aging

Mixed Biologic, Psychologic, and Social Sciences ($485,402)

Birren: Comprehensive Training Program in Gerontology
Maddox: Behavior and Physiology in Aging and Human Development

GENERAL

The Branch supports by reimbursable agreement with the Veterans Administration a series of yearly meetings to coordinate certain longitudinal studies on aging. This series of meetings was the mechanism finally worked out to coordinate longitudinal studies following a review of their problems requested by the House Committee on Government Operations. The management of these meetings is carried out by Dr. Benjamin Bell who is associated with the VA Normative Study of Aging. Representatives from this study and from a number of other longitudinal studies of aging attend these meetings, attempt to standardize their measurements so that data collected will be comparable, consider what areas in which pooling of data might be useful, and discuss what other problems they have in common.

NICHD has authority to make grants designated as Center Grants to institutions to facilitate, develop, and strengthen their capability for conducting an organized program of research on aging processes. Center Grants are concerned with support for scientific personnel and administrative management, common equipment, central support services, and development of new research areas and opportunities. NICHD policy has been to make such Center Grants only to institutions where a strong program of NICHD-supported research has been developed. Only one such award has been made and that to the Duke University School of Medicine.

THE USE OF EXPERIMENTAL ANIMALS IN AGING RESEARCH

Understanding of most of the aspects of the biology of man has been based to a large extent on studies of the biology of other living creatures. It appears likely that an understanding of the biology of aging in man will provide no exception to this general rule. A number of advantages can be expected to derive from the study of aging in other species. The study of aging can be compressed into a shorter period of time by studying species with a short lifespan. Experimental procedures can be undertaken that would not be permissible in man. Particular species may, through particular circumstances, highlight particular aspects of aging.

During recent years the extramural program has explored through conferences and personal exchange with investigators relevant factors in the selection and provision of suitable animals for experimental work on aging. The major effort has been to select mammalian species since their biology is so similar to that of humans. Most consideration has been given to the use of rats and mice since these are well studied animals, widely used by investigators in many fields. They are particularly useful in aging research since they have short lifespans and are small and thus relatively inexpensive to raise to old age. Dogs and nonhuman primates have been considered, but their size and long lifespans would seriously increase the cost and slow the pace of aging research.

In recent years methods have evolved that along with older methods permit the development of rodents well suited to aging research. Birth by Caesarean section and the use of barriers that exclude unwanted bacteria make it possible to raise rats and mice free of pathogens. In addition the genetic composition of the experimental animals can be controlled.

The contract mechanism has been used to establish colonies of aging rats and mice at the Charles River Laboratories. The animals are well defined genetically and with respect to their bacterial flora. Sample animals are autopsied at intervals so that they are also well defined pathologically. Their life-tables have been established on the basis of spontaneous mortality in the colonies. These animals can be purchased by investigators involved in aging research. The funds, of course, usually come from research grants.

However, even the shortest lived mammals live about three years. Thus research on aging in mammals tends to proceed at a slow pace. The only shorter lived animals are invertebrates, many of whom have lifespans measured in days. Since many of the cellular processes occurring in metazoa of all degrees of complexity are similar, it appears possible that important metazoan aging processes at a cellular level may be similar. For this reason investigators have sought invertebrate models for human aging. Several different invertebrates have been studied. Some particularly rewarding results have been found using a nematode (roundworm) with a lifespan of about 30 days. In these animals a progressive loss of the enzymatic activity per unit weight of enzymes has been found as a function of age. A search for a similar process in mice is now underway. If this succeeds, it will present an example of how exploratory work in lower life forms can accelerate our understanding of mammalian aging.

CELLULAR AGING

Early in the evolution of life the unicellular life forms that had developed probably did not reproduce sexually. Instead, each cell divided to produce two new cells. Thus there was no senescence. When these unicellular organisms evolved into multicellular animals (metazoa) then specialization led to the development of two major types of cells, the sexual cells and the somatic (body) cells. The species were maintained by the periodic fusion of sexual cells leading to the creation of new organisms composed of both sexual and somatic cells. These organisms, composed largely of somatic cells, carried the sexual cells until the reproductive process was repeated. This cycle was essential for the maintenance of the species. However, there was no necessity for the somatic cells of the individual organisms to survive indefinitely. Thus, in general, selective forces did not lead to the creation of immortal individuals. Instead the somatic components of organisms, the bodies that carry the sexual cells, evolved in such a way that they had limited lifespans. This was the evolutionary origin of senescence.

Once organisms composed of both sexual cells and body cells had evolved along with the senescence of the body cells, there was no necessity for individual immortality of body cells or cell lines. Cells can be classified as those that have lost the ability to divide (fixed post-mitotic cells) or as dividing cells. There are no known fixed post-mitotic cells with an indefinite lifespan. The situation is different for cell lines that continue to divide within the individual. The potential immortality of some types of somatic cells has persisted in some animals and disappeared in others. The persistance of potentially immortal cells is striking in some lower invertebrates - flatworms, for example. Simply by cutting individuals of this species in two they can be propagated indefinitely.

In the course of evolution, mammalian body cells, having no need for this potential immortality, may have lost it. At least, no mammalian body cell type has been discovered that is capable of indefinite propagation. This limitation of lifespan is not a necessary characteristic of mammalian cells. Many types of mammalian cancer cells are potentially immortal and can be transferred from host to host indefinitely. Some mammalian cells that have undergone certain transformations in tissue culture can also be grown indefinitely in such culture.

It appears probable that normal mammalian body cells age and die because they lack processes to repair the damage that they sustain as a result of their own metabolic processes or of adverse environmental effects. Certainly the development and genetic transfer of repair processes for the key deteriorative processes involved in senescence would have imposed a heavy genetic burden on any complex species. Progress has been made by a number of investigators, some Institute-supported, in recent years in the development of methods for studying several lines of replicating cells. All of these lines have a limited ability to propagate themselves. The normal human fibroblast can be grown in tissue culture. There it propagates itself by cell division. However, after about 50 cell doublings it loses the ability to divide and dies. Epithelial cell lines from mouse mammary glands can be transferred from young mice to young mice so that they live longer than the mice in which they originated. However, the cell lines die out after several transfers. The precursors of red blood cells can also be transferred from mouse to mouse. But, once again, they will not continue to propagate indefinitely. Institute-supported investigators are investigating the mechanisms that distinguish immortal from mortal cell lines. Investigations are more advanced in the study of human fibroblasts than in the other systems.

A promising approach involves experiments aimed at the environmental processes that may damage cells. Some of these processes that damage cell constituents involve reactive oxygen and chemical compounds known as free radicals. These processes are thought to be blocked by various antioxidants and there is suggestive, though not conclusive, evidence that antioxidants may prolong the lifespan of cell lines in tissue culture and of both invertebrates and vertebrate experimental animals.

ENDOCRINE CHANGE WITH AGE

Changes with function with increasing age occur in a number of the endocrine glands. The major programmatic efforts of the Institute in this area have dealt with the menopause and post-menopausal state.

The female reproductive system undergoes major changes during the course of its development and decay. After a period of relative inactivity during childhood, ovarian activity increases dramatically at puberty. The body produces eggs and hormones in a strictly patterned reproductive cycle which, unless interrupted by pregnancy, disease, or hormonal contraceptives, repeats itself with little variation until about the age of 50. At that time, and over a period of several years, ovarian activity falters and then almost completely ceases.

The hormones secreted by the ovaries at menarche and through the reproductive years exert a profound influence on the maturation and maintenance of the

breasts and sexual organs. The great reduction in ovarian excretion of estrogen during the menopause and in the post-menopausal years causes atrophic changes in tissues previously stimulated by estrogen.

Institute supported investigators are studying the mechanisms involved in the failure of the ovarian system and the hormonal changes that result as a result of that failure. It has been found that some adrenal hormones are converted into compounds with estrogenic activity in the peripheral tissues and help to reduce the severity of post-menopausal symptoms. Studies of the side reactions associated with the hormonal treatment of the menopause are in progress.

IMMUNOLOGIC AGING

The observation made 45 years ago that the serum concentration of natural antibody to A and B blood group antigens declines with age in man, is probably the earliest evidence of a decline in immune function with age. Discoveries in recent years have indicated the severity of this functional loss. Immune competence increases rapidly in the first years of life, reaches a maximum in the teens and then declines progressively so that in the elderly that competence may decrease to a tenth of what it was at its earlier peak. Since immunologically incompetent persons are very susceptible to infections and cancer, the immunological incompetence of the elderly is probably the source of many of their health problems. The possibility that this incompetence can be improved by medical treatment makes it a particularly important area for investigation.

Investigators supported by the Institute are studying the processes involved in the loss of immunologic competence. A conference was held this year to discuss the nature of the changes with age and to discuss future directions for research. A brief report of this conference was published.

Several major changes play a part in immunologic aging. There is a loss of antibody-forming cell precursors from peripheral lymphoid organs, changes in the architecture of the peripheral lymphoid tissue, and a generalized loss of thymus-derived cell function. Relatively early in life atrophy of the thymus begins and slowly progresses with age. Investigators have had some success in modifying the immunologic decay that occurs with aging in experimental animals. For example, caloric restriction has been shown to permit a much better retention of immunologic competence by rodents as they age.

COGNITIVE CHANGE WITH AGE

Studies of cognitive and intellectual changes with age and the morphological, physiological, and biochemical changes in the central nervous system which probably underlie these changes are of major concern to the Institute.

There are major changes in mental function across the years. Many of these changes are easily discernible by observation. Such observations have made us aware of the speed with which children learn, and forget, new languages in contrast to adults, the importance of early entry into many types of activity in determining later competence, the greater creativity of the years of late adolescence and early childhood and the senile dementias. These all attest to changes in mental function with age. However this knowledge is not the result

of controlled experimentation, and may be misleading. Uncontrolled and even unrecognized variables may be operating that make much of what we think we know about this field unreliable.

Most studies of intellectual competence as a function of age have been cross-sectional--that is, they have compared the function of individuals of different ages at the same point in time. These studies have shown a decline in intellectual capabilities with age. The declines in general have been small with tests of verbal ability and larger with tests of problem-solving ability. Recently, longitudinal studies have cast doubt on these findings. When the same individuals are tested at two points in time, making possible measurement of the decline in each of many different individuals, there may be little or no decline in the scores obtained on various tests that measure intelligence.

An exception to this occurs in some patients with demonstrable abnormalities. For example, in later life what is measured as intelligence has been shown to decline in subjects who have cardiovascular hypertension.

Recent studies are also pointing to the possibility that "training" older persons to develop test-taking strategies serves to reduce their anxiety regarding taking tests and they actually perform better on tests measuring intellectual abilities. Older people tend to lack experience with tests and this inexperience results in lack of cognitive skills and strategies and in a general test anxiety.

More studies need to be carried out in the area of intellectual decline with age. Studies showing no loss in intelligence have to be reconciled with what would be expected on a basis of the generalized brain atrophy that occurs with increasing age and with the fact that an appreciable fraction of older people have such severe loss of mental function that they are considered demented.

Senile dementia was once considered to be due mainly to cerebral areterioscleroosis. Most neuropathologists now think that this is not the case and that loss of function with age is a result of neuronal changes not dependent on vascular inadequacy. Most ot the patients with senile dementia and many with dementia occurring in the middle years of life have pathological changes usually considered characteristics of Alzheimers disease. However, it is not clear whether this is a disease or simply aging of the brain occurring usually late in life but occasionally somewhat prematurely.

Institute-supported investigators made several important discoveries during the year that provide leads for future research. It was shown that there is an appreciable decline in learning ability in rats as they age. They thus provide an experimental mammalian model for the study of cognitive change with age. It was also shown that there is a progressive loss of connections between the brain cells of aging rodents. This suggests at least one mechanism for their loss of cognitive ability.

Societal Aspects of Aging

The average duration of life has changed greatly since the evolutionary emergence of mankind, and there have been corresponding changes in the age structures of

the many different populations that have existed on the earth. There is no reason to think that these age-structures will not continue to change, and it is possible that they will be exaggerated by major changes in the average length of life. The human race has adjusted to a great variety of age-structures successfully in the past and must continue to do so in the future.

Although mankind has adjusted successfully to different population sizes and age-structures these demographic characteristics may provide major challenges to societal function in the future. The severity of these changes will be determined in part by future fertility and mortality rates.

Future changes in mortality rates will probably play a minor role in determining total population. For example, doubling the average lifespan would only double the population and it would require an average human lifespan for this to occur. Fertility is the major cause of population explosion, and on a global scale is doubling the population every 30 years.

Fertility rates are also very important in determining what fraction of the population is elderly. Ten percent of the population is now over 65 years of age. With zero population growth, current mortality rates, and no migration, this country would ultimately have 16 percent of its people over that age. This is scarcely more than the percentage of elderly people that some prosperous European countries have at present. Decreased mortality rates due to the control of vascular disease could increase the percentage of the elderly even more and create problems if the increase in lifespan were not accomplished by a substantial decrease in the deteriorative processes of aging. For this reasons the Institute supports research to investigate the implications of changes in mortality rates for society and the individuals who compose it.

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: HD-4-2811

Contract Title: Development of a Production Colony of Three Genotypes of Laboratory Mouse for Aging Research

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: $104,116.00 (FY 1975)

Objectives: 1. Provide characterized genetically defined strains of laboratory mice reared in a defined environment for research in aging.

2. Develop a ready commercial source of aging mice of three basic genotypes to meet the demands for aging laboratory mice.

3. Minimize lag time for the development of studies in aging requiring aged genetically defined laboratory mice from a controlled environment.

4. Provide the minimum number of strains of mice necessary for cross comparison and extrapolation of experimental results to a broader natural population.

5. Develop a colony of laboratory mouse strains in which pathological processes, degenerative change, morbidity and mortality to age 24 months are largely known and predictable.

Significance for Aging Research: A lack of aged genetically and biologically defined animals reared in a controlled environment has long hampered the development of aging research, particularly in the field of immunology. With increasing frequency studies in aging research require animals of known genetic background, biological characterization and environmental status. To meet this need for strains of genetic specificity, diversity and generalizability a colony of aging mice of the inbred strains C57/BL6, BALB/c, the inbred F_1 hybrid of the two inbred strains was established in a barrier enclosure (SPF) at Charles River Breeding Laboratories. Profile data will be acquired on the colony and strains of animals by periodic sacrifice and necropsy.

The major significance of this contract is the development of a readily available resource of aging, genetically defined and characterized strains of laboratory mice reared in a controlled environment. The standing colony of aging mice of the three genotypes proposed under this research contract provides investigators in aging with basic genetically controlled model systems previously unavailable to most investigators in aging. This has moderated one of the primary constraining influences on the development of aging

research in animals by making available: 1) basic genetic model systems of the aging laboratory mouse for studies in aging requiring specific genetic control, 2) for study, one or several comparative animal model systems within a species, 3) an animal of known biological characterization and environmental status.

Proposed Course: Contract will continue for a minimum of one year with the contract becoming increasingly self-sustaining in subsequent years as animals are provided to investigators for research on aging.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: 72-2767

Contract Title: Review, Analysis, and Evaluation of Models for Forecasting Future U.S. Population

Contractor: Duke University
Durham, North Carolina
(Contract Officer: Dr. George Myers)

Money Allocated: $68,165.00 (FY 1975)

Objectives: This contract supports a review that will identify existing population models as well as classify and evaluate them. The aim is to develop predictive models that will be responsive to current needs and provide more sophisticated approaches to population planning for the elderly.

Major Findings: The effects upon population of eliminating major causes of death have been examined. Four causes of death were treated: major cardiovascular-renal diseases, influenza and pneumonia, malignant neoplasms and motor vehicle accidents. The projected population age 65 and over for the year 2000 resulting from the elimination of major cardiovascular-renal disease was nearly 75% greater than what would have resulted if this disease had not been eliminated. The corresponding difference for the projection eliminating malignant neoplasms was only 14%.

Significance for Aging Research: Basic to the process of planning for the social, enonomic and health of the older population is an adequate understanding of its future size and composition.

Proposed Course: The contract is scheduled to terminate June 1975.

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: HD-2-2792

Contract Title: Prevention of Bone Loss in the Menopause

Contractor: Creighton University, Omaha, Nebraska

Money Allocated: $47,000.00 (FY 1972) (Extended Without Funds)

Objectives: The purpose of this project is to study prospectively the effect on calcium metabolism and on bone mass of supplementing the diet with increased calcium or of administering sex hormones to women at or after the natural menopause. The study group consists of seventy-five nuns varying in age from 50 to 65 years. They will be assigned at random to three equal groups. One group is given calcium carbonate, the second group premarin with methyltestosterone, and the third group is a control. At the end of the second year of the project, comparisons of regression of bone mass as a function of age in the three groups will be accomplished to examine differences in rate of loss of bone. The study may provide information on the mechanisms by which these differences occur.

Significance for Aging Research: This study represents one of the first reasonably accurate assessments prospectively of the effect of combined estrogen-androgen replacement therapy on bone resorption and formation in postmenopausal women. Data correlating the effects of treatment with estrogen-androgen replacement or oral calcium on bone in the menopause is highly significant to assessing the effectiveness and need for replacement therapy in postmenopausal women. Thus, the studies supported under this contract have significance for understanding the changes that occur in bone mass with aging and will provide important information on calcium metabolic changes that result from therapeutic intervention in postmenopausal women.

Proposed Course: The contract to study prevention of bone loss in the menopause will be completed early in FY 1976.

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: HD-4-2843

Contract Title: Production Colony of Aging Rats in an Isolator Environment

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: $47,627.00 (FY 1975)

Objectives: 1. Establish a production, rearing and maintenance system for aging rats that prevents introduction of pathogens prematurely shortening the natural lifespan of aging laboratory rats.

2. Determine the commercial and research feasibility as well as per animal cost effectiveness of isolator rearing of aging laboratory rats compared with barrier and conventionally reared rats.

3. To develop for research in aging:

a) a limited source of aging rats free of bacterial and detectable viral diseases.

b) a system of environmental control that is standardized and transferable to the research laboratory without unduly compromising the biological integrity of the animals.

c) laboratory rats that are known to survive to natural senescence as a natural population independent of bacterial diseases or detectable viral diseases.

4. Compare survival, pathology and degenerative change in isolator reared rats with barrier and conventionally reared animals.

Significance for Aging Research: The lack of aging experimental animals of defined quality is one of the major limiting factors to the study of aging, particularly animals that survive to natural senescence. Until recently aging studies in animals, especially rodents, were limited to those animals hardy enough to survive the stress of disease and a fluctuating physical environment. During the past several years several methods have evolved in the development, husbandry, maintenance and care of laboratory animals that permit routine cesarean derivation and rearing of laboratory rats and other experimental animals behind a barrier or in the rigidly controlled environment of plastic isolators. The isolator excludes the introduction of bacterial and detectable viral agents. This methodology combined with genetically defined animals on a stabilized diet and a close monitoring system goes far toward establishing and providing aging animals that survive to natural

senescence independent of the complications of infectious disease, parasitism, or wide variability in physical environment.

Basically the contract for an isolator reared colony of aging rats is concerned with developing an aging rat colony in a rigidly controlled isolator environment free of bacterial agents, except those purposefully introduced as normal bacterial flora. Changes in the aging process can most readily be determined when environmental conditions are uniformly controlled and sufficient numbers of animals representative of the total population of animals survive to an aged condition that can be studied as characteristic of the normal processes of aging. For this reason the contract requires uniformity in environment, diet, humidity and temperature and genetic quality of the animals in the isolator.

Once it is clearly established that laboratory rats can be maintained for their full lifespan independent of detectable microbial disease, and survival rates largely offset added costs for isolator maintenance, a resource of isolator reared animals can be provided to investigators in aging. The need for developing expensive and sophisticated environmentally controlled buildings to support the colony is unnecessary since the total environment of the animal is transferred within the isolator unit.

Studies of aging independent of environmental variables and disease can then be conducted to increase understanding of aging processes in animals and how these processes may apply to man or lead to experimentation in man. The major significance of the isolator system for rearing laboratory animals lies in the enhancement of detectable change in aging processes.

Proposed Course: The isolator contract is to be continued for a minimum of two years. Continuation will be dependent on commercial feasibility and colony survival rates.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: HD-3-2725

Contract Title: Contract to Breed, Rear and Maintain a Colony of Inbred Aging Laboratory Rats for Aging Research (Modified)

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: $97,854.00 (FY 1975)

Objectives:

1. Meet current and projected demands for senescent laboratory rats reared on a defined diet in a specific pathogen-free environment.

2. Establish a standing commercial resource of senescent rats on which investigators can immediately draw for aged laboratory rats.

3. Develop baseline physiological and pathological characterization of the Fischer 344 rat over their full lifespan.

4. Establish survival curves for laboratory rats reared specific pathogen-free behind a defined barrier system.

5. Increase the numbers and ages of animals to be made available for studies in aging.

Significance for Aging Research: A major constraint influencing the development of aging research has been the almost total absence of an aged animal resource sufficiently characterized to meet the unique needs of aging research. The development of a colony of aging laboratory rats under this contract will significantly enhance the quality and quantity of aging research by providing aged animals that are reared in a defined environment on a standardized diet, free of pathogenic organisms, and characterized with regard to age-specific causes of death.

Basic to the development of studies in aging research in animals is a characterization of expected physiological and pathological changes that may occur over the animals' full lifespan as well as life tables that accurately reflect survival at specific ages. A primary aim of this contract is to acquire this data and make it available to investigators in aging. With this information, a reasonable comparative assessment can be made as to whether the animals, strain or stock is suitable for studies in aging. Also, within reasonable limits, numbers of animals needed for statistical significance of studies can be readily established, thus minimizing the likelihood of supporting excessive numbers of animals or too few animals for statistical significance of the study.

Currently many investigators in aging cannot acquire aged animals short of rearing the animals themselves, nor are they able to maintain aging rats under the laboratory conditions necessary to allow the animals to survive long enough to observe truly senescent change with age. Also, competent young investigators more often than not cannot support aging colonies of rats until they successfully compete for research support. Without this resource many imaginative young investigators will continue to be excluded from research in aging simply because they are unable to identify an aged animal resource which they could use in the studies they propose in aging.

Proposed Course: Contract is to be continued for a minimum of two years with the contract becoming increasingly self-sustaining, and until age-specific causes of death and life tables for the colony and strain are established.

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: NO1-HD-4-2865

Contract Title: Selection, Production, Characterization and Distribution of Genetically Marked Cells for Aging Research

Contractor: Institute for Medical Research, Camden, New Jersey
(Contract Officer: Dr. Warren W. Nichols)

Money Allocated: $5,850.00 (FY 1975)

Objectives: To establish and maintain a repository of frozen viable genetic and mutant cell cultures that are of interest in aging research and to hold a workshop addressing the use of genetically marked cells for aging research.

Significance to Aging Research: The in vitro expression of genetic uniqueness of different cell strains offers powerful means to investigate mechanisms of aging at the cellular and subcellular level. This contract is to encourage the use of somatic cell genetics in studies of cellular aging, provide characterized, contaminant-free cell cultures to qualified investigators, and enhance research through communicative activities such as the annual workshop. The Institute for Medical Research resource is to be a focal point of grant program activities in cellular aging.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: 72-2755

Contract Title: Quantitative Studies of Aging Human Diploid Fibroblasts *in Vitro*

Contractor: University of Vermont
(Contract Officer: Dr. Marlene Absher)

Money Allocated: $47,744.00 (FY 1975)

Objectives: The Contractor is describing the division patterns and cellular lineages of human cells grown in culture utilizing time-lapse cinematographic, autoradiographic and computer analysis and model simulation techniques.

Significance for Aging Research: The human diploid cell in culture is a widely-studied model for aging. Populations of these cells double actively under standard cell culture procedures for many months, but eventually age and die. Although extensive research is being conducted on populations of such cells, the studies are being pursued without definitive knowledge of the division characteristics of individual cells and their progeny. There is evidence that "old" populations contain many "young" behaving cells, just as "young" populations contain "old" behaving cells no longer capable of division. These and other data are being produced by Dr. Absher to the end of refining experimental design, cellular aging research concepts and hypotheses, and a capacity to relate cell culture studies of aging to the aging process in man.

Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: Unassigned

Contract Title: Aging Monkey Tissues and Organ Resource

Contractor: Washington, State University, Pullman, Wash.

Money Allocated: $10,869.00 (FY 1975)

Objectives: 1. To acquire organs and tissues from a rare resource of six (6) aged rhesus monkeys ages 24 to 26 years as the animals become moribund or expire.

2. To select and preserve organs and tissues from each of the animals that are or may be required for the study and inter-species comparison of aging and aged changes in the Rhesus monkey and other mammalian species.

3. To bank fresh, frozen or chemically fixed and preserved tissues and organs from each of the six monkeys as they become moribund or expire.

4. To provide selected tissues and organs on request for studies in aging.

Significance for Aging Research: The study of aging requires the availability of tissues and organs from a wide variety of strains and species of animals. To study aging changes and the comparative differences between the ordered lifespan of different species of mammals requires that the program identify and develop resources that meet the needs of the investigator in aging research. Preservation and provision of tissues and organs from aged subhuman primates, essentially expiring from natural causes, will provide a continuing resource of rare and unique materials that would otherwise be lost to aging research. The contract essentially supports the complete postmortem evaluation and preservation of tissues and organs of each of the six monkeys as they become moribund and/or expire. Postmortem protocol will require that all tissues and organs be examined, classified, and characterized. Tissues and organs from all major body systems and the integument will be selectively preserved based primarily on the requirements of the individual investigators. Other tissues and organs will be preserved by freezing or fixed in chemicals as well as preparation of slide sets of tissues from major organ systems. These materials can be provided on request for study of aging changes in the sub-human primate or comparative studies between species.

Proposed Course: Contract is to be continued for a minimum of two years.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: HD-4-2756

Contract Title: Origin and Action of Estrogen in the Postmenopausal Woman

Contractor: The University of Texas Southwestern Medical School, Dallas, Texas

Money Allocated: $110,000.00 (FY 1975)

Objectives: The long-range goal of this project is to determine qualitatively and quantitatively estrogen production in postmenopausal women and the mode of interaction of estrogens and their target tissues. Earlier studies have shown, in oophorectomized postmenopausal women, that the principal estrogen produced is estrone and that for the most part, this arises from the extragonadal, extra-adrenal aromatization of androstenedione. In normal postmenopausal women most of the androstenedione is secreted by the adrenal glands but in women with ovarian stromal hyperplasia it can be secreted by the ovary as well. Also, changes in estrone have been shown to be associated with a variety of factors including obesity, age, diabetes, hypertension, liver function and some ovarian neoplasms. In view of the acquisition of this earlier data, the primary objectives of the contract are aimed at:

1. Establishing the relative contributions of ovaries and adrenals to plasma androstenedione and the extent of conversion of androstenedione to estrone and estrone sulfate prior to and following oophorectomy.

2. Determining the chemical nature of the hormone product following aromatization of androstenedione.

3. Delineating the effect of obesity, aging, hypertension, diabetes, liver function and some ovarian neoplasms on the conversion of androstenedione to estrone and estrone sulfate.

4. Determining the capacity of a variety of non-endocrine tissues to achieve the conversion of androstenedione to estrone by *in vitro* incubation studies.

5. Evaluate the relative efficacy of substances potentially active as aromatase inhibitors in an *in vitro* system utilizing a placental aromatizing enzyme system to inhibit excessive conversion of androstenedione to estrone, that occurs in some postmenopausal women.

6. Examining the interaction of estrone and estradiol in target tissue such as human endometrium and immature rat uterus.

With the attainment of these contract objectives, the origin and action of estrogen in postmenopausal women can be clearly defined as well as quality

and quantity of estrogen production.

Significance for Aging Research: The results of this study should contribute to understanding of the physiology and pathophysiology of estrogen production after the menopause as well as its medical management. Neither the quantitative nor qualitative characteristics of estrogen production in the menopause have been fully elucidated. It is clearly evident that the data on levels of endogenous estrogen production, the chemical nature of estrogens produced and their biological activity as well as factors which may contribute to alterations in endogenous estrogen production are needed. The acquisition of data under this contract is critical to understanding the degree of decline in estrogen production and the alternative methods by which estrogen production occurs in postmenopausal women. Also, a rational approach to replacement therapy in postmenopausal women cannot be developed until quality and quantity of endogenous estrogens produced during the menopause are clearly delineated.

Proposed Course: The contract will be concluded at the end of FY 1975.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: HD-3-2762

Contract Title: A Retrospective Study of Postmenopausal Women with and Without Estrogen Replacement Therapy

Contractor: University of California at Irvine, Irvine, California

Money Allocated: $147,383 (FY 1975)

Objectives: The aim of the proposed epidemiologic study is to determine whether and to what extent estrogen usage tends to increase the risk of stroke in a population of postmenopausal women living in a retirement community. The contract provides for the support of a retrospective study of the incidence of cerebrovascular disease, and other factors which predispose to cerebrovascular disease in a specific and uniquely discrete population of 7,000 postmenopausal women. Cases of cerebrovascular disease occurring in postmenopausal women will be identified and described (morbidity and mortality). The population of identified stroke cases will be compared with an appropriate control group to determine if the case group is significantly different from that of the controls. Also, to determine the role of likely risk factors, proximity, dose and duration of drug usage in affecting severity, or type of stroke.

Significance to Aging Research: Until recently, the long-term effects of estrogens in postmenopausal women have been a matter of conjecture or largely ignored. The proposed study will provide current data on comparative risk of cerebrovascular disease in postmenopausal women taking estrogens and postmenopausal women who are not using them, but are otherwise at comparable risk from other causes.

The significance of this project lies in the fact that a substantial percentage of the women in the population are taking estrogen-like medications and the initial goal of the study can be completed within a two and one-half year period. At present the value of estrogen-like medication as therapy for postmenopausal symptoms, for the prevention of vascular disease, and for the arrest of osteoporosis remains unproven. There is serious concern that such medication in commonly used dosage is a significant health hazard with specific reference to the three most common causes of death--heart disease, cancer, and stroke. There are no data available on the questions posed by this proposal. The population selected is uniquely constructed to permit this type of investigation. Information of this type is extremely important, since it may serve to guide the medical care of the many millions of women over the age of 50 in the United States.

Proposed Course: The study is planned for a minimum of one year. Continuation is dependent on results from the first two years' study.

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Gerontology Research Center

Office of the Chief

The entire Intramural Research Program of the NIA is located at the Gerontology Research Center, Baltimore, Md. This GRC report therefore represents the report of all Intramural scientific activities of the Institute.

The program of the GRC is organized under four laboratories and Branches, viz., Laboratory of Behavioral Sciences, the Clinical Physiology Branch, Laboratory of Cellular and Comparative Physiology, and the Laboratory of Molecular Aging. The Office of the Chief provides research services (technical development, electronic data processing, photography and arts, animal resources, and library services) to all operating Branches and Laboratories as well as supervision of the Collaborative Guest Scientist Program.

In FY 1975 29 visiting scientists, visiting fellows, and guest scientists with 34 supporting personnel worked in GRC laboratories in addition to the 154 budgeted positions. Another 58 employees in part-time, temporary, WAE, stay-in-school, and work-study categories participated in the GRC programs.

Research Services

The Technical Development Section has continued to maintain and service scientific equipment for all investigators at the GRC. The Section has collaborated with the Cardiovascular Section in the design and construction of an instrument which can mechanically stretch papillary muscles, isolated from rat hearts, in the order of microns at frequencies up to 100 Hz while recording milligram levels of resulting tensions. With this equipment critical experiments on the effects of aging on heart muscle can now be pursued. To meet the demand for increased on-line capability for recording and processing data, an additional central processing unit was purchased and interfaced with recording instruments located in specific laboratories. Software was also developed to allow easy implementation of smaller computers and microprocessors as on-line satellites to the central computer. These additions have greatly improved data collection and analysis for the research programs.

The Animal Resources Facility provided housing and care for approximately 10,000 rats, 13,000 mice, 150 guinea pigs (strain 13), 100 rabbits (2,000 rabbits were received and issued), 35 senescent beagles, 100 hamsters, and 8 monkeys to support research programs at the GRC. Senescent (2-year-old) rats and mice as well as matching controls at 3 mo., 6 mo., 12 mo., and 18 mo. are now available to investigators on a regular basis.

During the year the GRC Public Information Office handled 293 public inquiries about aging. In addition, 107 contacts were made with news media, 258 people were given guided tours of the GRC, and 1,938 publications were distributed. Studies conducted at the GRC were reported in various newspapers, periodicals, radio, and television presentations. Arrangements were made for the VA to film sequences in the GRC Laboratory of Behavioral Sciences as part of a training film entitled "Dialogue on Biofeedback".

In addition to providing library services to GRC investigators, the Library staff continued to search out and index the world literature relating to all aspects of aging.

Collaborative Guest Scientist Program

During the year 14 programs were supported by the GRC. These programs were staffed by 20 investigators with 34 supporting personnel.

Cardiac Performance in the Rat. --The previously reported age-associated prolongation of the duration of contraction was not related to the ability of cardiac muscle of rat to develop tension under isometric conditions nor the maximal rate of tension development. This age change may be related to prolongation of the active contractile state of the contractile element rather than alterations in the passive stiffness or viscous properties of cardiac muscle. The prolonged active contractile state may be due to either increased calcium affinity of the contractile proteins or an age-associated alteration in the active cardiac relaxing system. The age-associated decrease in the inotropic response to norepinephrine does not appear to be a result of an increased uptake of norepinephrine nor to an inability of heart muscle of old animals to respond to any inotropic stimulus. The combination of the age decrease in inotropic response and the persistent shortening of contraction duration results in a marked decrease in the ability of catecholamines to augment peak tension developed under isometric conditions. Exposure of cardiac muscle to hypoxia results in a similar depression of performance during hypoxia in muscles from young and senile rats. However, during reoxygenation the muscles from the older age group demonstrated a significantly greater prolongation of contraction duration during the early post-hypoxic period. These results again suggest that there is an age-associated alteration in the sarcoplasmic reticulum with age. These studies were carried out in collaboration with scientists of the GRC.

Cell Biology. --Endogenous norepinephrine levels were lower in the hearts of old than in young rats. Age differences in the norepinephrine uptake by the neuronal axoplasm could not account for these decreased levels. There was an age-associated increase in turnover rates of norepinephrine which was accompanied by normal rates of catecholamine synthesis. Studies of the subcellular distribution of intravenously administered ^{3}H-norepinephrine showed an inability of the myocardium of aging rats to retain norepinephrine in the storage granules with subsequent loss to the soluble fraction. This storage defect was partially corrected by pretreatment with mercurial diuretic suggesting that altered ionic distribution within, or in the vicinity of, the sympathetic neurons may play an important role.

Earthworms make a useful model for the study of the factors which control proliferative growth in animals. The variation in earthworm regeneration was shown not to be due to the manner of wound healing nor the method of transection. Several different patterns of simultaneous and repeated anterior and posterior transections demonstrated that the factors responsible for setting the limits of regeneration are indeed localized within the tissues adjacent to the cut surface and are not hormonal in nature.

Effect of Bacterial Enterotoxins. --An age-related stimulation of cyclic AMP-dependent lipolysis in rat epididymal fat cells due to products of V. cholerae and E. coli has been investigated. An enhanced effect following the administration of epinephrine has been demonstrated in toxin-treated cells. This altered hormone response has been related to a soluble component of cytosol. This soluble activity is affected by toxin treatment of the whole cell. In the presence of a high concentration of fresh cytosol toxin effects can be demonstrated in a cell-free system.

Laboratory of Behavioral Sciences

The Laboratory of Behavioral Sciences carries out studies which describe in quantitative terms the behavioral changes that take place with age. Efforts are made to determine the basic mechanisms of these changes. Furthermore, techniques and programs are developed to mollify or eliminate the psychologically mediated impairments and disabilities of the aged.

A collaborative study between investigators in the Learning and Problem Solving Section and investigators from the Clinical Physiology Branch on the interaction of ethanol and age has been completed. These studies have shown that ethanol more severely impairs old (55-80 years) than young (20-54 years) subjects on a number of important behavioral measures including reaction time and memory. The older subjects not only were more severely impaired acutely, but also recovered more slowly.

Studies from other laboratories have shown cohort as well as age effects in a variety of measures of intellectual performance. Cohort effects arise because persons born during a particular period will have unique experiences which are attributable to the socio-cultural milieu in which they are reared. Therefore, longitudinal analyses of behavior must be controlled for cohort effects. Studies of longitudinal changes of memory and learning in this Laboratory have shown that there are age-related deficits in mental performance. Men who were 69 to 76 years old at the time of first measurement showed the greatest decline in mental performance on retesting. Studies of groups of men with equivalent birth dates (i.e., cohorts who were tested at different ages) have shown that the cohort sample which was older when first tested performed less effectively on several tests of learning and memory. The differences among cohorts were such that the declines in performance were greatest for the earliest born cohorts. Impairment in both longitudinal and cohort samples was similar; older subjects committed more errors on first testing. Furthermore, older subjects increased their errors significantly more than did younger subjects on retesting after a 7-year

interval. Therefore, whether age comparisons are made longitudinally, cross-sectionally, or within cohorts, the results are the same, i.e., learning and memory decline with age, and the decline is greatest for the oldest men.

Studies of problem solving in which learning and memory factors are minimized are showing important age-environment interactions. On the one hand, laboratory studies of age differences in concept problem solving have shown declines after age 60. These findings replicate previous cross-sectional findings in this Laboratory. On the other hand, well practiced subjects in their 60s, i.e., subjects who have had the opportunity to solve hundreds of problems, attained a level of performance similar to that of young adults. Furthermore, even subjects in their 90s are showing that they too can solve these difficult problems when they are well practiced.

The Learning and Problem Solving Section has shown that longevity is significantly related to body weight, diet, and exercise. Among rats which were not allowed to exercise, about 40% (22 of 60) failed to survive to age 22 months. Among rats given ad lib access to run-wheels from age 45 days, only 8% (2 of 24) were dead at 22 months. In a study of two inbred strains of mice and their hybrid cross, animals which received a low (4%) protein diet showed a greater degree of survival than did animals which received a normal (26%) protein diet. The percentages of mice which reached advanced old age (based on strain-specific norms) on low protein diets were 34% for one strain, 26% for the other strain, and 42% for the hybrids. On normal protein diets survival was 10%, 4%, and 10%, respectively, for these strains. Among five genetically identifiable groups of mice, the non-mutant control animals (C57BL/6J) lived about 14% longer than did two light weight mutant groups, and about 35% longer than did two very heavy mutant groups. However, the higher the peak weight among individual animals, the longer they survived relative to their group expectancy. Furthermore, it has been shown that animals with the longest growth period, i.e., the greatest age of attainment of peak weight relative to their norms, survived longest.

Two studies designed to identify the physiological and behavioral mechanisms of learned cardiac control have been completed on monkeys. In one study of the physiological mechanisms of such learning, it was shown that animals have characteristic heart rates below which they cannot slow. The relationship between degree of slowing and baseline heart rate is linear. When the animals were given the vagolytic drug, atropine, their ability to slow heart rate was reduced; however, they still were able to slow. When the animals were given the sympatholytic drug, propranolol, the ability to raise heart rate was enhanced. Behavioral studies of animals have shown that when animals are required to speed their heart, they tend to increase general activity whereas when they are required to slow heart rate they tend to decrease activity. Nevertheless, within any particular animal, the ability to slow or to speed its heart was not correlated with activity. Thus, animals apparently establish "sets" to speed or to slow heart rate; however, once set, the control of heart rate appears to operate independently of body movement.

Clinical Physiology Branch

The Clinical Physiology Branch has continued to emphasize research on aging processes in man, but with important supplementation by studies in the rat. The Baltimore Longitudinal Study of Aging provides the primary resource for studies in man and efforts have continued not only to provide basic data on longitudinal age changes in individuals but also to probe into the underlying mechanisms of these changes.

The eighth 2-year cycle of tests in the Longitudinal Study will be completed on June 30, 1975. The basic statistics of the study as of December 31, 1974 are: (1) 1012 Ss seen at least once; (2) 615 Ss have had at least 4 examinations and 238 Ss have had at least 8 examinations (older Ss are seen annually); (3) there have been 135 deaths since the study was initiated in 1958; (4) in addition to the deaths, a number of subjects have become too ill to return, some have retired and moved too far away to be seen every 2 years, and some have inevitably lost interest--636 subjects are currently active participants; (5) the active participants are well represented throughout the 25-84 year age span. The unique logistic feature of this study is the fact that subjects spend 2 1/2 days with us on each visit. This enables us to undertake numbers and types of tests which are not possible in other longitudinal studies.

We noted on last year's report plans to introduce seven new procedures. The follow-up on these plans is as follows: (1) Echocardiography is now a routine part of the testing program; (2) a comprehensive protocol for the study of sex hormone function has been approved by the Human Research Committee and methodologies have been nearly completed; (3) skin fibroblast cultures have been obtained on young and old adults and show significant age differences as reported elsewhere--the preliminary results are so promising that characterization of cells in culture in more of our participants, including middle-aged subjects, will be continued; (4) post-heparin triglyceride lipase release was successfully completed; (5) urinary 24-hour sodium excretion is now a routine measurement and correlations with blood pressure will be started; (6) retinal photography has just been approved by the Human Research Committee and photography will start soon; technician has completed training at the Wilmer Eye Institute at the Johns Hopkins University; interpretation of films will be made at the Wilmer Institute in collaboration with us; (7) the ethanol infusion study was completed. In general, while not all goals were met, progress has been satisfactory.

An analysis of longitudinal trends in activity level and in body weight reveals essentially three phases in adult life. In the early adult years (25-44), weight tends to increase as activity level falls. In the late years (65-84), weight falls and activity levels also decline. An interesting characteristic of the 55-64 age group is that this is the only group in which subjects increase their activity level as they age and weight is maintained during that decade. This is also the decade when a large fraction of subjects retire (mean age of retirement in these subjects is 64 years). The activity spurt may represent the sudden availability of leisure time to a group of men who have been in predominantly sedentary occupations.

It is also of interest that there are differences in the mortality experience of subjects who did or did not participate in regular exercise. The sedentary group died 2 1/2 years earlier than the active group and the causes of death were different, with a larger percentage of the sedentary group dying of vascular diseases (69%) than of the active group (44%), while deaths from cancer were 18% and 44% in the two groups.

The decline of sexual activity in the later years has been quantified in the Longitudinal Study participants. While multiple factors undoubtedly underlie this age change, very little objective information is available. The careful multidisciplinary assessment of aging characteristics in this study now allows for the examination of some of these factors. Some variables which might have been expected to correlate with the level of sexual activity had no discernible effects--for example, certain estimates of general physical fitness such as activity calories, vital capacity, forced expiratory volume at one second, grip strength, lean body weight. In the oldest age group (65-79 years) modest but significant correlations did occur with chest circumference, maximum breathing capacity, the BMR, and serum cholesterol level. There is also a strong tendency for subjects to maintain their relative levels of sexual activity over many years. An important negative correlation was that prostatic size was unrelated to the level of sexual activity; there had been much speculation on this point.

Scientists in the Clinical Physiology Branch selected two drugs, both metabolized by the liver, but by different mechanisms, to assess age changes. Alcohol was chosen because of its widespread use, its physiological effects, and the profound clinical, legal, and social problems involved in its use. The other drug, antipyrine, was picked because it represents a drug family metabolized by another basic liver mechanism. Both drugs were given intravenously to volunteers whose chronological ages covered the adult life span. The study shows that alcohol is metabolized as well by older men as it is by younger men. On the other hand, the ability to dispose of antipyrine decreased with age. An analysis of data on smoking and coffee or tea drinking habits collected routinely on members of the longitudinal group suggests a possible reason for the slower metabolism of antipyrine in older men. Both habits, regardless of age, speed up antipyrine metabolism. However, cigarette smoking and coffee or tea drinking decline in older volunteers. This indicates that much of what appears to be a decrement related to biological aging may be attributed more to age-associated changes in habits.

The relation of the function of endocrine organs to aging has been studied by several techniques. It has been found that glucocorticoids inhibit tritiated uridine uptake of rat splenic leukocyte and this inhibition decreases with age. However, these data are difficult to interpret since the various types of splenic cells continually replicate. Therefore, recent studies have been performed on organs whose cells essentially do not replicate after early life. Thus studies on neurones from the rat cerebral cortex and adipocytes from rat epididymal fat pads were carried out. In these two cell types glucocorticoid effects (stimulation of ^{3}H-norepinephrine uptake in neurones and stimulation of lipolysis in adipocytes) are decreased with age. There has also been direct demonstration in both cell types of a decrease in receptors for the hormone. Parallel studies have been conducted on another

system on cell membranes. Several hormones exert their effects by stimulating the membrane enzyme, adenylate cyclase, leading to the production of cyclic AMP. Detailed studies of aging effects in rat adipocytes show distinct effects of three hormones (epinephrine, glucagon, and ACTH). The decline with age is greatest for glucagon so that by 12 months no effect remains. The other two hormones show age effects but they are much less striking and responsiveness remains into old age. These studies are now being extended by examining human fat cells removed at surgery from volunteers. Early studies show a remarkable insensitivity to catecholamines unless the nucleotide Gpp (NH)p is added.

Another complexity of this area is that cell growth during aging in the rat could lead to decreased sensitivity by the effect of the increased cell surface area on which the hormone receptors are located. Hormonal sensitivity was studied by examining the effects of glucagon on lipolysis in adipocytes. Cell size was controlled by introducing caloric restriction both to young rats as they aged to prevent increase in cell size and to older rats to reduce enlarged cells to the size of those younger rats. The diminished hormonal sensitivity of the cells of the older rats could neither be prevented nor reversed.

The isolated trabeculae carneae preparation from young adult and old hearts continues to be a valuable preparation for further investigation into the mechanism of the deterioration of cardiac function with aging. Digitalis is a therapeutic agent widely used in the treatment of heart failure in the elderly. It is known to increase the force of the cardiac muscle contraction (inotropic effect). A rapidly acting digitalis preparation, ouabain, was therefore evaluated by several techniques. Its inotropic effect is decreased with age, but the aged myocardium can respond normally if a higher dose is employed. There is thus not a change of capacity of the heart, but of sensitivity to the drug. Supporting this concept is the fact that the inotropic effects of paired pacing (electrical stimulation) do not decrease with age. Furthermore, measurements of binding of ouabain to the membrane showed a decrease with age. Ouabain acts biochemically by inhibiting membrane Na-K ATPase activity; this drug effect is also lower in the old rats.

Laboratory of Cellular and Comparative Physiology

The Laboratory of Cellular and Comparative Physiology conducts studies on the nature of the age-related deterioration of certain cells of the immune and related systems, determines the underlying cellular and molecular mechanisms responsible for this deterioration, and develops methods for early detection of signs of cellular aging and methods to control or reverse these changes.

Studies conducted on stem cells, which are the precursors of T cells, indicate that the proliferative capacity of old stem cells grown in old mice is only about 10% that of young stem cells grown in young mice. This difference is due to age-related changes in the cellular environment and age-related changes within the stem cells. Recent studies showed that, although the differentiation capacities of thymic tissues following mitogenic stimulation

generally decline gradually with increasing age, a few decreased rather abruptly early in life. These results indicate that certain subpopulations of T cells are generated only during development whereas others are generated throughout life.

Since early differentiation events following antigen or mitogen activation of lymphocytes occur at the level of the cell membrane, studies on the effects of age on membrane structures have been pursued systematically. The results revealed that lymphocytes of old mice appear to be more fragile than lymphocytes of young mice. In addition, enriched T cell fraction from young mice respond to T cell-specific mitogen, PHA, by generating a cyclic -GMP level four times higher than that of old mice. Finally, cyclic-AMP can stabilize lymphocyte membranes.

Studies on molecular etiology of aging, which were initiated recently, center about the effects of aging on the fidelity of B cells. Antigen-induced specific antibodies of IgG_1 and IgG_2 isotypes obtained from adult (3 month) and aged (2-3 years) inbred guinea pigs are being analyzed by various physicochemical parameters. The present results indicate that the differences appear to be minimal. If these preliminary studies can be verified, they would suggest that B cells do not seem to undergo marked changes with age, confirming the earlier observations based on cellular analysis of aging mice.

Studies have proven both by direct and indirect methods that macrophages can differentiate adult self from senescent self on the basis of selective immunoglobulin G isotype attachment to the surface of senescent cells. Young red blood cells (RBC) were aged in vitro by pretreating them with neuraminidase. These artificially aged RBC were phagocytized as rapidly as in situ aged RBC. This suggests that carbohydrate moieties are being exposed as RBC age naturally and, once exposed, these receptors are bound by pre-existing autoantibody-like immunoglobulins. The results indicate that the molecular basis of recognition of senescent cells by macrophages is immunological.

Rejuvenation of old mice, as assayed by regaining much of the reduced immunologic activities, can be brought about by the combined treatment of infusion of young stem cells and implantation of newborn thymic tissue, or by injection of 2-mercaptoethanol, a reducing agent.

Serially passaged culture studies revealed that as human fibroblasts age in vitro they become bigger, heavier, and less proficient in their ability to proliferate. The large cells were distributed in all phases of the cycle. Furthermore, the variation in DNA content of individual cells increased. These characteristics were induced in mitotically active, small young fibroblasts by culturing them in serum-deficient medium and by exposing them to hydroxyurea, an inhibitor of cellular proliferation. It would seem, therefore, that an increase in cell size and density may be a manifestation of cells becoming less proficient in their proliferative ability.

A study on maternal age effects was initiated a year ago to develop the mouse as an animal model to investigate the effects of maternal age on chromosomal disorders. The results indicate that with increased maternal

age the frequency of chromosomally abnormal fetuses increases from 3% to 15%. Mosaicism and trisomies are the most frequently observed forms of chromosome abnormality. A good correlation has also been observed between morphologically abnormal fetuses and chromosomal abnormalities.

Evidence shows that the specific activity of purified aldolase declines with age. This is associated with an increase in heart lability. Peptide mapping studies show that differences can be detected between young and old liver aldolase. Immunological analyses of the purified enzyme preparation show that the old enzyme preparation contains two antigens, one of which is immunologically identical to the young liver aldolase. It remains to be established if the second antigen contains enzyme activity.

Laboratory of Molecular Aging

This Laboratory has given attention to two critical areas known to undergo perturbations that lead to the inability of organisms to maintain homeostasis, viz., physiological control systems and genetic information transfer systems. These studies have impact on the mechanisms of age-dependent alterations in the following physiological systems: renal function, skeletal muscle activity, cardiac function, and metabolism.

The kidney is an organ which permits unique opportunities for studies on the molecular basis of the aging process. First, renal function itself is altered by age, and second, in other illnesses, e.g., congestive heart failure, renal adjustments to maintain fluid and solute homeostasis are slower in the aged. Techniques have been developed to isolate the luminal (brush border) region of the proximal tubule plasma membrane in the form of osmotically active vesicles. The membranes are used as a model system to study mechanisms by which solutes are transported from the glomerular filtrate across this membrane into the cell. Kinetic studies of the Na^+-dependent D-glucose uptake by membranes indicate that the action of the Na^+-gradient can be separated into a stimulatory effect of Na^+ when D-glucose and Na^+ are on the same side of the membrane and an inhibitory effect of Na^+ when the sugar and Na^+ are on opposite sides of the membrane. Other studies suggest that the Na^+-dependent transport of sugar by the renal tubule is an electrogenic process.

The maintenance of acid-base balance by the kidney is critically important to the aged, especially when stressed. The three main acidifying processes, bicarbonate reabsorption, the generation of titratable acid, and ammonia production are thought to be mediated by the common mechanism of H^+ secretion. Considerable interest has focused on the tubular mechanism underlying H^+ transport. Significantly, we now report the presence in isolated luminal membranes of an ATPase which is activated by bicarbonate. As a working hypothesis, it is proposed that, in analogy with other membrane ATPases, hydrolysis of ATP by the renal membrane is coupled to the expulsion of protons into the tubular lumen. Protonation of HCO_3^- in the filtrate results in increased CO_2 which is then transported across the membrane.

It has been previously reported that mitochondria in muscle (blowfly

flight muscle) in situ show age-related membrane damage and these ultrastructural alterations are correlated with biochemical decrements in the maximally stimulated (ADP-induced) oxidative phosphorylation system. It is now found that mitochondria from aged blowflies show similar decreases in rates of uncoupled respiration. This finding suggests that the age-dependent defect lies within the oxidative or electron transport pathway, and is not associated with phosphorylation. Assays of partial reactions of electron transport and contents of cytochromes further localize the site of the age-dependent defect. The specific activity of the "latent" ATPase was increased significantly in mitochondria from senescent blowflies. Perhaps of considerable significance are lipid analyses of flight muscle mitochondrial membranes showing that mitochondria with the greatest decline with age are those deficient in Vitamin E, a natural component of membranes presumably having a role as an antioxidant.

An essential step in understanding the mechanisms by which heart muscle contractibility changes with age is to define the transport processes which are basic to regulation of cardiac function. One system being investigated is the transport and intracellular compartmentation of Ca^{2+} by the sarcoplasmic reticulum. A preparation of sarcoplasmic reticulum has been obtained from rat heart and analyses of the rate of Ca^{2+} uptake as a function of concentration suggest that the uptake mechanism is second order with respect to Ca^{2+}. An initial comparison of uptake by 6 and 24-month-old rats shows that V_{max} remains constant but that Km decreases in the older population. This apparent decrease in affinity for Ca^{2+} is consistent with an increased contraction duration observed in isolated aged myocardium of rats.

It has been previously demonstrated that divalent metal ions in concentrations not much higher than those required for biological processes can induce mispairing of nucleotide bases. Mispairing can produce errors in every phase of information transfer, in replication, transcription, and translation. NMR studies have shown that the mispairing phenomenon can be reversed by raising the temperature. The new experiments reveal that the errors produced by the metal ions can be countered by other parameters. Ribosomes from different species have different degrees of fidelity in translation, when challenged by metal ions. Experiments have shown that such differences can result from differences in the ability to counteract the effects of the metal ions.

An increase in concentration of altered proteins in aged cells has been described. If this increase is due to errors in protein synthesis, challenge of these cells with virus should result in a corresponding increase in altered viral proteins. Such a challenge of WI38 cells was carried out with polio virus. No proportionate change in viral proteins was observed. It is concluded, therefore, that the alterations are not due to errors in protein synthesis. Post-transcriptional changes in the proteins can better explain all of the results.

NICHD Annual Report
July 1, 1974 through June 30, 1975
Gerontology Research Center
Clinical Physiology Branch

The Clinical Physiology Branch has continued to emphasize research on aging processes in man, but with important supplementation by studies in the rat. The Baltimore Longitudinal Study of Aging provides the primary resource for studies in man and efforts have continued not only to provide basic data on longitudinal age changes in individuals but also to probe into the underlying mechanisms of these changes. The challenge to design such experiments has been successfully met in some areas in man; in other cases, especially in the endocrine and cardiovascular areas, isolated tissue preparations have proven to be valuable.

The eighth 2-year cycle of tests in the Longitudinal Study will be completed on June 30, 1975. The basic statistics of the study as of December 31, 1974 are: (1) 1012 Ss seen at least once; (2) 615 Ss have had at least 4 examinations and 238 Ss have had at least 8 examinations (older subjects are seen annually); (3) there have been 135 deaths since the study was initiated in 1958; (4) in addition to the deaths, a number of subjects have become too ill to return, some have retired and moved too far away to be seen every 2 years, and some have inevitably lost interest--636 subjects are currently active participants; (5) the active participants are well-represented throughout the 25-84 year age span. The unique logistic feature of the study is the fact that subjects spend 2 1/2 days with us on each visit. This enables us to undertake numbers and types of tests which are not possible in other longitudinal studies.

We noted on last year's report plans to introduce seven new procedures. The follow-up on these plans is as follows: (1) Echocardiography is now a routine part of the testing program; (2) A comprehensive protocol for the study of sex hormone function has been approved by the Human Research Committee and methodologies have been nearly completed; (3) Skin fibroblast cultures have been obtained on young and old adults and show significant age differences as reported elsewhere--the preliminary results are so promising that characterization of cells in culture in more of our participants, including middle-aged subjects, will be continued; (4) Post-heparin triglyceride lipase release was successfully completed; (5) Urinary 24-hour sodium excretion is now a routine measurement and correlations with blood pressure will be started; (6) Retinal photography has just been approved by the Human Research Committee, and photography will start soon; technician has completed training at the Wilmer Eye Institute at the Johns Hopkins University; interpretation of films will be made at the Wilmer Institute in collaboration with us; (7) The ethanol infusion study was completed. In general then while not all goals were met, progress has been satisfactory.

In addition to these new efforts, analyses have continued on ongoing studies. An analysis of longitudinal trends in activity level and in body weight reveals essentially three phases in adult life. In the

early adult years, weight tends to increase as activity level falls. In the late years (65-84 yr) weight falls and activity levels also decline. An interesting characteristic of the 55-64 age group is that this is the only group in which subjects increase their activity level as they age, and weight is maintained during that decade. This also is the decade when a large fraction of subjects retire. The activity spurt may represent the sudden availability of leisure time to a group of men who have been in predominantly sedentary occupations. It is also of interest that there are differences in the mortality experience of subjects who did or did not participate in regular exercise. The sedentary group died 2 1/2 years earlier than the active group and the causes of death were different, with a larger percentage of the sedentary group dying of vascular diseases (69%) than of the active group (44%), while deaths from cancer were 18% and 44% in the two groups.

An analysis of seasonal differences in the dietary habits of the subjects showed differences only for vitamin A, C, and B6, with lower intakes in the winter months. This was unexpected in this upper-middle socio-economic group. Intakes for other nutrients did not show these seasonal trends. This general similarity of dietary intake through the seasons simplifies analysis of such variables as serum lipid levels and glucose tolerance which could have been influenced by significant dietary seasonal shifts.

Automation of the measurement of carbon dioxide metabolism and ventilation volume during arm exercise tests has been accomplished and longitudinal study of our subjects show increases in metabolism per unit of work, i.e., a decrease in the efficiency of movement. Ventilation volume also increases. Rate of recovery from the effects of the exercise is also decreased with age. With the automation we should be able to measure gas exchange during the treadmill exercise test; this is a much harder exercise load and these measurements will therefore provide a measure of cardio-respiratory fitness.

The decline of sexual activity in the later years has been quantified in the Longitudinal Study participants. While multiple factors undoubtedly underlie this age change, very little objective information is available. The careful multidisciplinary assessment of aging characteristics in this study now allows for the examination of some of these factors. Some variables which might have been expected to correlate with the level of sexual activity had no discernible effects--for example, certain estimates of general physical fitness such as activity calories, vital capacity, forced expiratory volume at one second, grip strength, lean body weight. In the oldest age group (65-79 yr) modest but significant correlations did occur with chest circumference, maximum breathing capacity, the BMR, and serum cholesterol level. There is also a strong tendency for subjects to maintain their relative levels of sexual activity over many years. An important negative correlation was that prostatic size was unrelated to the level of sexual activity; there had been much speculation on this point.

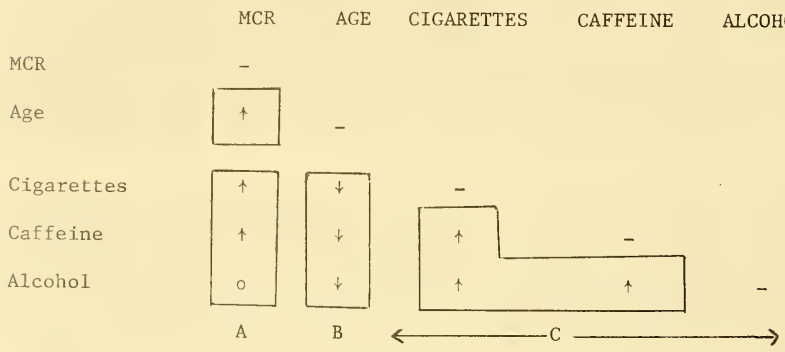
A series of studies has been completed and partly analyzed on the possible role of lipoprotein lipase to the known differences in serum lipid levels with age. Lipase is liberated into plasma by the injection of small amounts of heparin intravenously. The lipase was separated into extrahepatic (protamine inactived, PI) and hepatic (protamine resistant, PR) sources. Age had no effect on either of these moieties in 50 Ss and thus lipase differences do not explain the differences in level of circulating lipids. In 38 subjects, heparin was administered at the conclusion of the intravenous alcohol infusion test. Again no age differences in PI or PR were found. Interestingly, alcohol did cause an increase in serum triglyceride levels and the increase was positively correlated with age. However, older subjects had higher peak ethanol levels, and when this factor was taken into account, no age difference in triglyceride response to ethanol remained.

Collaboration with Dr. John C. Brown of the Department of Physiology, University of British Columbia, has been established for the evaluation of the role of "gastric inhibitory polypeptide" (GIP) in the economy of glucose by the body. GIP is a hormone produced by cells of the small intestine and released when glucose is ingested. It stimulates the release of insulin from beta cells of the pancreas. The glucose clamp technique permits quantification of the role that this newly discovered hormone might play in the glucose intolerance of aging, of obesity, and of diabetes mellitus. The studies are conducted on Longitudinal Study participants using the "hot hand" techniques; the warming of the hand in a heated box arterializes the venous blood leaving the hand as we showed previously. The immunoassay for GIP is accomplished by Dr. Brown. Results show serum GIP and insulin curves of remarkable similarity following glucose ingestion under conditions of constant, maintained hyperglycemia. This is a clear demonstration that endogenously released GIP in man is an important mediator of insulin secretion. The presence of a wide range of ages, degrees of obesity, and degrees of glucose intolerance in the Longitudinal Study will permit evaluation of these interrelated variables with respect to the role of GIP.

While there are a number of scattered observations in the medical literature on the effects of age on drug effects, on drug blood levels, and on drug toxicity, a remarkable deficiency of carefully conducted studies of age effects on the pharmacokinetics of drugs exists. Such studies on two drugs have now been completed. (1) Antipyrine was given in the Longitudinal Study initially as a indicator for total body water in evaluating body compositional changes with age. Since it was given in a controlled fashion intravenously, its rate of disappearance could be computed. (2) The choice of a second drug was based upon a number of considerations: the drug selected should be acceptable to volunteers, it should be clinically important and in common use, it should be assayable, and it should have effects which could also be measured. We chose ethanol as a drug which admirably possessed those qualities.

The age effects on these two drugs revealed interesting facets of the complex clinical pharmacology of aging. Antipyrine was administered

to our participants without regard to certain "habits" which could induce alterations in drug metabolism (cigarette smoking, ethanol and caffeine ingestion). It was therefore possible to analyze for the effects of these habits as well as for the effect of age. The results can be summarized in the following table in which the metabolism of antipyrine is expressed as the metabolic clearance rate (MCR):



These complex interrelations can be understood if they are considered in groups. Consider Group C in the Table: These arrows indicate that cigarette smoking, the ingestion of coffee and tea, and alcohol usage are all positively correlated. Thus an apparent effect of caffeine could be secondary to the tendency for the high caffeine group also to be the high cigarette group. Group B shows that each of these habits is significantly lowered with increasing age. Group A demonstrates that by simple paired correlation, MCR is significantly higher in cigarette smokers and in caffeine users. The arrows in the single box indicates that MCR decreases with age. To dissect out the primary role of aging and of these habits on MCR it was necessary to use multi-variate analyses. In essence these show that a large part of the effect of age on drug metabolism is secondary to the difference with age in smoking habits. Cigarette smoking accounts for about 15% of the total variance in MCR, age for 3%, and the other habits have no independent effect. The importance of these studies lies in the fact that studies of effects of aging in man must take into account many differences among the age groups studied. Thus some age effects are not due to primary biologic processes of aging but are due to differences in the habits of people of different ages.

The studies of alcohol metabolism have provided information which is basic to an understanding of the effects of this drug. In order to study pure physiological processes of drug distribution and metabolism in the body, the alcohol was given as a constant intravenous infusion over a one hour period. The dosage was equivalent to ingesting 3 single high-balls. The blood alcohol levels achieved were higher in the older subjects despite the fact that dosage per unit of body surface area was the same for

all subjects. This reflects a change in body composition with age; the total body water content per m^2 surface area decreases as cellular mass is lost with increasing age. The metabolic clearance rate of this drug however is unchanged with age; the drug disappears from the blood stream just as rapidly in older subjects as in younger. The effects of alcohol were measured in several ways. Effects on intellectual functioning and on reaction times are reported by the Laboratory of Behavioral Sciences. The effect of alcohol on hypothalamic-posterior pituitary function was assessed by immunoassay for the antidiuretic hormone arginine vasopressin (AVP). Plasma levels of this hormone were suppressed by alcohol; this is the first direct demonstration of this effect. Furthermore there were distinctive age differences in this effect. As the alcohol blood level rose during the one hour infusion, the AVP level fell progressively in young subjects and then returned to the basal level as the alcohol was metabolized. In older subjects the suppressive effect of alcohol on the hypothalamus was not sustained: during the second thirty minutes of infusion the AVP levels began rising despite a continual increase in blood alcohol levels and a marked rebound in AVP above the basal level occurs. Because of these surprising differences, further studies of hypothalamic responses have been investigated by the infusion of hypertonic saline as a stimulus to the secretion of AVP. These studies should help in the understanding of the increased susceptibility to serious disturbances in salt and water equilibrium in older patients.

The relation of the function of endocrine organs to aging has been studied by several techniques. In order to maintain hormonal function with increasing age, a number of processes must be considered; (1) the sensitivity of the endocrine gland to its normal stimuli must be maintained, (2) the receptors for the hormone on the surface of the cells of the target organs or intracellularly must be normal in quantity and quality, (3) the receptor-transducer-catalytic complex must be intact.

The field is very complex and results must be interpreted with caution as can be illustrated by studies on rat splenic leukocytes. It has been found that glucocorticoids inhibit tritiated uridine uptake and this inhibition decreases with age. This age effect could be due to (1) loss of splenic cells, (2) loss of protein content of cells with age, (3) changes in the population of cell type with age, or (4) selective loss of glucocorticoid-sensitive cells with age. Furthermore, (5) the actual age of the splenic cells may differ with age. Lastly, (6) there could be actual molecular cellular changes with age, i.e. a change in hormone-binding receptor quantity or quality. While the first three possibilities have been ruled out, it is impossible in this preparation to separate factors (4), (5), and (6). Recent studies therefore have been performed on organs whose cells essentially do not replicate after early life. Thus studies on neurones from the rat cerebral cortex and adipocytes from rat epididymal fat pads rule out factor 5. Furthermore all cells are hormonally responsive and factor 4 can be eliminated. In these 2 cell types then glucocorticoid effects are decreased with age (stimulation of ^{3}H-norepinephrine uptake in neurones and stimulation of lipolysis in adipocytes). There has also been direct demonstration in both cell types of a decrease in receptors to the hormone.

Parallel studies have been done on the second messenger system on cell membranes. Several hormones exert their effects by stimulating the membrane enzyme, adenylate cyclase, leading to the production of cyclic AMP. Detailed studies of aging effects in rat adipocytes show distinct effects of three hormones (epinephrine, glucagon, and ACTH). The decline with age is greatest for glucagon so that by 12 months no effect remains. The other two hormones show age effects but they are much less striking and responsiveness remains into old age. These studies are now being extended by examining human fat cells removed at surgery from volunteers. Early studies show a remarkable insensitivity to catecholamines unless the nucleotide Gpp(NH)p is added. These studies open a promising new area of gerontologic endocrinology in man since they permit study of the effect of age on the sensitivity of human tissues to hormones.

Another complexity of this area is that cell growth during aging in the rat could lead to decreased sensitivity by the effect of the increased cell surface area on the hormone receptors of the cell membrane. Hormonal sensitivity was studied by examining the effects of glucagon on lipolysis in adipocytes. Cell size was controlled by introducing caloric restriction both to young rats as they aged to prevent increase in cell size and to older rats to reduce enlarged cells to the size of those younger rats. The diminished hormonal sensitivity of the cells of the older rats could neither be prevented nor reversed; in that sense the age change is not artefactual.

The isolated trabeculae carneae preparation from adult young and old hearts continues to be a valuable preparation for further investigation into the mechanism of the deterioration of cardiac function with aging. Digitalis is a therapeutic agent widely used in the treatment of heart failure in the elderly. It is known to increase the force of the cardiac muscle contraction (inotropic effect). A rapidly acting digitalis preparation, ouabain, was therefore evaluated by several techniques. Its inotropic effect is decreased with age, but the aged myocardium can respond normally if a higher dose is employed. There is thus not a change of capacity of the heart, but of sensitivity to the drug. Supporting this concept is the fact that the inotropic effects of paired pacing (electrical stimulation) do not decrease with age. Furthermore, measurements of binding of ouabain to the membrane showed a decrease with age. Ouabain acts biochemically by inhibiting membrane Na-K ATPase activity; this drug effect is also lower in the old rats.

Previous studies had demonstrated a prolongation of muscle contraction with age which could be explained by delayed calcium removal from the muscle cell after contraction, thus prolonging the active state of the muscle. In order to investigate the intracellular mechanisms underlying these changes techniques have been developed for preparing the subcellular fraction, sacroplasmic reticulum, and this preparation is capable of taking up calcium in both the young and old heart. These studies are in progress.

Serial No. Z01-AG-00001-05-CPB
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individiual Project Report
July 1, 1974 through June 30, 1975

Project Title: The kinetics of glucose and insulin metabolism in man

Previous Serial Number: HD-CP-7

Principal Investigators: Elizabeth A. McGuire
Jordan D. Tobin
Reubin Andres
Mones Berman*

Other Investigators: J. Harold Helderman

Cooperating Units: *National Cancer Institute
Baltimore City Hospitals

Man Years:

Total:	3.3
Professional:	1.7
Other:	1.6

Project Description:

Objectives: These studies aim toward a more precise description of the complex interrelationships of glucose and insulin in man and an understanding of the nature of changes occurring in this regulatory system in states of altered carbohydrate metabolism (e.g., age, obesity, diabetes mellitus, uremia).

The focus of current studies is the control which insulin exerts on glucose production and utilization in states of normal and altered carbohydrate metabolism. The modeling effort is also directed toward a description of the role which glucose plays in insulin secretion in these same normal and abnormal states.

Methods Employed: A clamp technique is used which breaks the glucose regulatory feedback loop by maintaining blood glucose at a predetermined, constant level despite alterations in plasma insulin. Thus, glucose is raised to, and maintained at, a level above basal (hyperglycemic clamp) and the resultant changes in plasma insulin are determined. Alternatively, glucose is maintained at basal values while insulin is elevated through exogenous infusion (euglycemic-hyperinsulin clamp) allowing a

study of the effects of insulin on glucose metabolism without the added complexity which would have been encountered had insulin-induced hypoglycemia been permitted. Blood glucose is kept constant by a variable IV infusion of glucose. Blood glucose concentration is measured at 5 minute intervals by a rapid, automated method and appropriate changes are made in the glucose infusion rate to maintain blood glucose within ±5% of the desired concentration. Under conditions of constant blood glucose, the amount of exogenous glucose infused is a measured of net glucose utilization.

Injection of a tracer dose of glucose-1-^{14}C with determination of blood glucose specific activity (corrected for metabolites and recycling by established procedures) for 90 minutes prior to the start of the clamp permits determination of the parameters of the basal glucose model. Alterations in glucose specific activity during the euglycemic-insulin clamp period provide information on glucose production as it is affected by changes in plasma insulin concentration.

The data available from these studies are steady state data from the basal, fasting state (blood concentrations of glucose and insulin, and ^{14}C-glucose decay following a tracer dose) and transient data from the perturbed system (plasma glucose and insulin concentrations, glucose and insulin infusion rates, and ^{14}C-glucose specific activity).

These data can be used to describe the kinetics of the distribution and metabolism of glucose, the distribution and metabolism of insulin, and the control which each exerts on the other in the basal and hyperglycemic states. The modeling is done on a UNIVAC 1108 computer using the SAAM program of Berman and Weiss.

Major Findings: During the past year two refinements have been made in the glucose model. First, two paths of glucose production have been added to the model. One is negatively controlled by insulin and the other is independent of insulin control under the conditions of these studies. Second, the model has been revised to allow for the sampling of blood from either an artery or a vein. Arterialized venous blood (the heated hand technique) is a special case of venous sampling in which the concentration and time course of a substance are closer to, but not identical with those seen in arterial blood. This more general model permits the experimenter to use arterial or arterialized venous sampling without the necessity of different model parameters for each type of study.

In addition, a study was performed to investigate further the action of insulin on glucose utilization. Plasma glucose specific activity was determined for 90 minutes following injection of a tracer dose of glucose-I-^{14}C. A second tracer dose of glucose-I-^{14}C was given 120 minutes after the start of a euglycemic-hyperinsulin clamp and the decay of glucose specific activity was followed for 90 minutes more while the insulin clamp was continued.

Modeling of these data indicated that insulin may act on glucose utilization at two sites. The first and major action is a positive control of glucose utilization i.e., glucose loss from the system. In addition, insulin may control one of the exchange constants between plasma and an extravascular glucose compartment. This second site of insulin action cannot be resolved properly by the data presently available.

Euglycemic-hyperinsulin clamp studies utilizing the D-glucose-I-^{14}C technique have now been performed in 23 subjects. Of these, 16 were non-diabetics ranging in age from 18 to 78 yr and in obesity from 3 to 35% over "desirable weight"; 7 were diabetics, 21 to 78 yr of age and 5 to 32% overweight. Modeling of these data to examine the effects of these variables is under way.

Significance to Bio-Medical Research and the Program of the Institute: The remarkable prevalence (50%) of abnormal glucose tolerance tests in the older population of the United States coupled with the increased morbidity and mortality of patients with diabetes mellitus demands a delineation of the effects of aging on the pathophysiology of carbohydrate metabolism. These studies in man define kinetic parameters in the glucose-insulin homeostatic system and will apply such analysis to patients with disorders in that system.

Proposed Course of Project: Altered physiological and pathological states (age, obesity, diabetes, fasting, etc.) will be studied.

Keyword Descriptors: Aging, glucose, metabolism, insulin metabolism, glucose kinetics, insulin kinetics.

Honors and Awards:

Dr. Andres was invited speaker at Symposium on "Clinical Assessment of Aging" at the 10th International Congress of Gerontology, Jerusalem, June 1975.

Dr. Andres was presented the Kleemeier Award of the Gerontological Society at the Annual Meeting of the Society, Portland, Oregon, October 1974.

Dr. Andres chaired the Clinical Medicine Section Sessions on Endocrine Disorders at the 10th International Congress of Gerontology, Jerusalem, June 1975.

Dr. Andres delivered the Jesse C. Coggins Fund Lecture at the 177th Annual Meeting of the Medical and Chirurgical Faculty of Maryland on May 1, 1975.

Dr. Andres lectured at the 22nd Postgraduate Course of the American Diabetes Association, Chicago, January 30, 1975.

Dr. Tobin was invited speaker at the Symposium on "Endocrine and Aging" at the 10th International Congress of Gerontology, Jerusalem, June 1975.

Dr. Andres was invited speaker on Aging Processes and on Future Research Needs in Aging at the Symposium on "The Effects of Aging in Man" at the 22nd Annual Meeting of the American College of Sports Medicine, New Orleans, May 24, 1975.

Dr. Tobin was invited speaker on "The Endocrine System in Aging at the Symposium on "The Effects of Aging in Man" at the 22nd Annual Meeting of the American College of Sports Medicine, New Orelans, May 24, 1975.

Publications:

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3. Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The effect of age on the gastrointestinal mediation of insulin release

Previous Serial Number: HD-CP 27

Principal Investigators: Dana K. Andersen
Jordan D. Tobin
Reubin Andres

Other Investigators: John C. Brown*

Co-operating Units: *Department of Physiology
University of British Columbia
Vancouver, British Columbia, Canada

Man Years:

Total:	3.0
Professional:	1.4
Other:	1.6

Project Description:

Objectives: This study was designed to examine generally the influence of the gastrointestinal tract upon the insulin response to ingested glucose, and specifically the role of one or more gastrointestinal hormones in the process of insulin secretion. The study is being applied to altered physiologic states, including aging diabetes mellitus, and obesity. The observation that the response of the endocrine pancreas to ingested glucose is greater than its response to glucose administered intravenously, is well established. The physiologic and biochemical etiology of this finding, however, has remained open to debate and investigation. Similarly, the significance of this process in pathologic states is unknown. While gastrointestinal hormones have been suggested as possible mediators of the phenomenon, until recently none has been clearly shown to play a critical role. The development of a sophisticated laboratory technique for studying this process, and the availability of a specific assay for a newly discovered gastrointestinal peptide which appears a likely candidate as the soughtafer mediator, have provided the essential means of investigating this process.

Methods Employed. The hyperglycemic and euglycemic clamp techniques have been used to maintain human subjects at preselected elevated or normal blood glucose levels. This allows evaluation of factors affecting insulin secretion in the absence of changing blood glucose concentration. In addition, by maintaining stable glucose concentrations before and after ingestion of glucose solutions, the effect of the gastrointestinal influences may be specifically quantified. This allows precise determination of the sensitivity of endocrine cells in the gastrointestinal tract, the sensitivity of the pancreatic beta-cells to the gastrointestinal hormone(s), and the factors which affect the response of these tissues.

The hyperglycemic clamp study is performed by administering a calculated intravenous glucose load sufficient to raise the blood glucose level to a specific desired level. By using an automated, rapid blood glucose analysis technique, changes in the intravenous infusion are made every four minutes to insure a stable blood glucose level. After one hour of intravenous glucose infusion alone, a standard dose of an oral glucose solution is ingested by the subject, and the subsquent blood glucose level is maintained at the previous level.

In the euglycemic clamp study, the subject's normal, fasting blood glucose level is maintained by means of a combined insulin and glucose infusion, with the glucose infusion varying as before depending on the blood glucose concentration. The insulin infusion is given as a primed-continuous infusion, designed to provide a rapid square-wave elevation of insulin to a preselected level. After one hour of maintaining stable glucose blood levels, the standard oral glucose dose is given, and the euglycemic levels are maintained.

The subjects for study are primarily members of the Longitudinal Study, supplemented by some volunteer participants. The subjects are classified according to previous glucose tolerance (both oral and intravenous), age, obesity, medical and family history. Both insulin and gastric inhibitory polypeptide (GIP) are determined by radioimmunoassay of plasma samples. Lyophilized plasma samples are saved for further hormone assays.

Major Findings: In response to ingested glucose, both insulin and GIP are secreted rapidly. The insulin levels in the hyperglycemic clamp study show that the response to orally-administered glucose is roughly three times that of intravenous glucose, when blood glucose levels are approximately 220 mg%. GIP remains at baseline levels during intravenous glucose infusion, suggesting that little if any stimulus to the peptide producing endocrine cells is attributable to elevations in blood glucose levels within physiologic limits. Following oral glucose, GIP is rapidly secreted, reaching significantly higher levels within 10 minutes. A major observation has been that the GIP elevation appears to precede the insulin elevation by approximately 5 minutes; both hormones reach peak levels at the same time during the hour after oral glucose ingestion, and their plasma concentration curves are impressively

similar. These findings increase the evidence for their physiologic association.

While aging has been shown to result in decreased insulin responsiveness to glucose (administered both orally and intravenously), no data have previously been presented regarding the possible role of the gastrointestinal mediation of this process. The association with aging of the GIP response is currently being examined.

Obesity has similarly been known to affect the insulin response to glucose administration, with higher insulin levels seen after both oral and intravenous glucose. GIP appears to be similarly associated with obesity, in that higher GIP levels are seen initially, and the response to oral glucose is greater than in lean, age-matched individuals. The possible role of intestinal endocrine cell sensitivity to glucose in obesity is now being examined. Studies in diabetes are also proceeding.

Data obtained from the euglycemic clamp studies suggest that in the presence of normal blood glucose levels, there is no augmentation of insulin secretion after oral glucose administration. This suggests the interesting possibility of a "threshold function" of GIP's affect upon the pancreatic beta cell, with insulin response to GIP directly dependent upon the circulating blood glucose concentration.

Significance to Bio-Medical Research and the Program of the Institute: The high prevalence of altered glucose tolerance in aging and obesity, as well as the high incidence of adult-onset diabetes mellitus require further understanding of factors which contribute to this process. In addition, an understanding of similarities and differences in the pathophysiology of the various categories of glucose intolerance provides the hope for more and improved methods of treatment. Pathological states associated with alterations in gastrointestinal hormones are only superficially understood currently, and further investigation of these hormone systems adds greatly to a young area of medical knowledge.

Proposed Course of Project: Alterations in normal physiology (aging, obesity), metabolism (diabetes), and other pathological conditions (peptic ulcer disease, surgical intervention in gastrointestinal continuity) will be studied. Further elucidation of the physiologic processes in question will be examined in healthy subjects.

Keyword Descriptors: Aging, glucose metabolism, insulin secretion, gastrointestinal hormones, gastric inhibitory polypeptide.

Honors and Awards: None.

Publications:

DeFronzo, R. A., Cooke, C. R., Andres, R., Faloona, G. R., and Davis, P. J.: The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. J. Clin. Invest. 55: 845-855, 1975.

Serial No. Z01-AG-00003-03-CPB
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individial Project Report
July 1, 1974 through June 30, 1975

Project Title: Uremia and Carbohydrate Intolerance

Previous Serial Number: HD-CP 20

Principal Investigator: Ralph A. DeFronzo

Other Investigators: Reubin Andres
Jordan D. Tobin
John W. Rowe
Dan Sapir*

Cooperating Units: Johns Hopkins Hospital
*Renal Division

Man Years:

Total:	.10
Professional:	.05
Other:	.05

Project Description: This project has been completed and is being submitted for publication.

Proposed Course of the Project: Project is completed.

Keyword Descriptors: Uremia, glucose tolerance, insulin, growth hormone.

Honors and Awards: None.

Publications: None.

Serial No. Z01-AG-00004-02-CPB
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Ethanol metabolism.
A. The effect of age on the pharmacokinetics and pharmacologic effects of ethanol in man
B. Effect of uremia on ethanol metabolism and on the activities of the ethanol-oxidizing enzymes.

Previous Serial Number: HD-CP 30

Principal Investigators: Robert E. Vestal, Esteban Mezey*, John W. Rowe, James J. Potter*

Other Investigators: Elizabeth A. McGuire, Mones Berman**, Jordan D. Tobin, Reubin Andres

Co-operating Units: *Alcohol Research Unit, Baltimore City Hospitals
**National Cancer Institute

Man Years:

Total:	1.20
Professional:	4.5
Other:	7.5

Project Description:

Objectives: A. The purpose of this study was to examine under conditions of acute ethanol administration the effect of age on: (1) the kinetics of distribution and elimination of ethanol, (2) insulin, carbohydrate and lipid metabolism, (3) posterior pituitary function and free water clearance, (4) psychomotor and cognitive performance.

B. Uremia is known to be associated with disturbed elimination of several drugs which are metabolized by the liver. Since alcohol is metabolized primarily by an unique hepatic mechanism, the present study was designed to determine the effect of experimentally induced uremia on rates of ethanol disappearance from the blood, and on the ethanol oxidizing enzymes.

Methods Employed: A. Ethanol was administered intravenously as a 15% solution in a dose of 400 mg/m^2 surface area/min over 60 minutes following an overnight fast. Blood ethanol concentrations were

measured by gas liquid chromatography at fixed intervals during and for 4 hours after the infusion. The SAAM-26 computer program is being used to develop a compartmental model to describe the kinetics of distribution and elimination.

B. Studies were conducted in 21 male Sprague-Dawley rats. Uremia was surgically produced in 13 of these animals by removing approximately 85 percent of the renal mass. Eight animals served as sham-operated controls. In vivo ethanol disappearance rates from the blood were determined. In addition in vitro assay of enzyme activities for alcohol dehydrogenase, catalase, microsomal ethanol oxidizing system, aniline hydroxylase, N-demethylation, cytochromes P-450 and b_5, and cytochrome c reductase were carried out on liver cell homogenates. Blood urea nitrogen in all animals was determined prior to sacrifice. Because of a recently published report correlating alcohol dehydrogenase activity and ethanol disappearance rates with ascorbic acid levels in human leucocytes of chronic alcoholics, plasma and leucocyte ascorbic acid levels were also measured in some animals.

Major Findings: A. Studies in 47 subjects, age 21 to 81, have been completed. Rates of ethanol metabolism are not influenced by age. A highly significant negative correlation of ethanol distribution volume with obesity index (ratio of actual to desirable body weight) was observed ($r = -0.60$ $p<0.001$). Positive correlations of similar magnitude ($r = 0.67$, $p<0.001$) were present between total distribution volume and body weight. Thus, surface area did not provide a better estimate of distribution volume than did body weight. Peak ethanol levels were 14 percent higher in the older subjects ($p<0.001$).

B. A three-fold increase in plasma urea nitrogen was observed in the animals. The mean hepatic alcohol dehydrogenase activity was increased almost two-fold in the uremic rats. There were no changes in the activities of the other enzymes measured. The increase in alcohol dehydrogenase activity could not be reproduced by incubation of liver from a normal rat with uremic rat plasma, with uremic human serum or with urea. The increase in enzyme activity was not associated with changes in leucocyte ascorbic acid levels or in ethanol disappearance rates.

Significance to Bio-Medical Research and the Program of the Institute. A. Aging has been associated with decreased metabolism of several drugs. The metabolism of ethanol differs from that of most other drugs in that it is oxidized principally by an enzyme in the soluble function of liver cell homogenates, and only to a lesser extent by the microsomal enzyme fraction. Our results indicate that age does not influence ethanol metabolism, but that it may influence ethanol distribution due to differences in body composition.

B. Since uremia is associated with decreased elimination of some other drugs metabolized by the liver and with decreased activity of liver microsomal enzymes, this observation is an unique and unexpected

finding. Further investigation will be necessary to elucidate the physiologic significance and the mechanism for the increase in alcohol dehydrogenase activity in uremia.

Proposed Course of Project: The studies are completed. However, additional analysis of the human data is being carried out in order to explore further the relationship of ethanol distribution to differences in body composition and to differences in peak ethanol levels. This analysis to some extent awaits completion of the modeling of the data using the SAAM-26 program. This analysis will be completed by July 1, 1975.

Keyword Descriptors: Age, ethanol kinetics, uremia, ethanol metabolism, liver enzymes.

Honors and Awards: None.

Publications:

Mezey, E., Vestal, R. E., Potter, J. J., and Rowe, J. W.: Effect of uremia on rates of ethanol disappearance from the blood and on the activities of the ethanol-oxidizing enzymes. J. Lab. Clin. Med. (in press).

Serial No. Z01-AG-00005-02-CPB
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Hypothalamic-Hypophyseal Responsiveness and Aging

Previous Serial Number: New Project

Principal Investigators: J. Harold Helderman
Robert E. Vestal
Jordan D. Tobin
Reubin Andres

Other Investigators: Gary L. Robertson, M. D.*

Cooperating Units: *Veterans Administration Hospital
Indiana University School of Medicine
Indianapolis, Indiana

Man Years:

Total:	1.5
Professional:	0.8
Other:	0.7

Project Description:

Objectives: These studies are designed to ascertain the effect of aging on hypothalamic-hypophyseal secretion of the antidiuretic hormone, arginine vasopressin (AVP), in response to: 1) overnight dehydration, 2) hypertonic saline infusion and 3) intravenous ethanol infusion. From such studies mechanisms of aging changes will be described and some of the clinical problems in salt and water conservation in aging individuals may be explained.

Methods Employed: The hormone arginine vasopressin was measured by radioimmunoassay of acetone-extracted plasma utilizing the Glick-1 (Gl-1) antiserum. The assay can be performed on sample volumes of 1 ml or less and is sensitive at 0.5 pg/ml of the hormone. The technique is highly specific as it can distinguish AVP from all other naturally synthesized and secreted posterior pituitary peptides. Samples of plasma obtained during the various perturbations were rapidly frozen and assayed at a later date.

In all studies subjects across the age spectrum were carefully

to eliminate those who had cardiac or renal diseases and those taking alcohol or drugs. They refrained from fluids overnight, a period that averaged 10.3 hours. Plasma samples obtained just prior to a perturbation thus represented the AVP response to mild overnight dehydration. In one group of studies 3% NaCl was infused by vein over 2 hours following a 20 minute basal period in the recumbent position. Plasma samples were obtained at 20 minute intervals during the infusion for plasma AVP, sodium, and osmolality. Urines were collected before and after the infusion for the measurement of sodium and osmolality. The goal of these studies is to provide a hyperosmolar stimulus which is recognized by the hypothalmus and leads to posterior pituitary release of antidiuretic hormone.

In another group of studies 15% (v/v) ethyl alcohol was infused intravenously at the rate of 400 mg/m^2/min over one hour (an amount equivalent to three martinis). Blood samples for AVP, ethanol, sodium, and osmolality were obtained at intervals over 5 hours. Urines were voided in one group ad libitum and at fixed intervals in another group. As ethanol has been implicated in diminishing or abolishing secretion for AVP, it provided a negative stimulus to test the hormonal axis.

Urine and plasma measurements of osmolality and volume allowed computation of osmolar clearance (Cosm) and free water clearance which quantify the physiologic response to the AVP present in the blood.

Major Findings: After a mild overnight dehydration there was no difference in the AVP level between younger and older subjects. Basal plasma osmolality, serum sodium, free water clearance, and Cosm were all similar in the two groups.

Intravenous ethanol inhibits the secretion of the antidiuretic hormone in all subjects as suspected. Aging alters the hormonal response to ethanol. Younger subjects demonstrate an inhibition of AVP secretion in response to the ethanol reaching a nadir at the end of the infusion of 55% of the baseline values. Older subjects respond initially reaching a nadir of 71% of baseline half way through the infusion. The AVP levels then paradoxically rise back to the baseline despite continued elevation of ethanol in the blood. The hormone in the older group then rebounds to nearly twice basal levels at the conclusion of the study.

Free water clearance was negative after an overnight dehydration; the subjects were concentrating their urine, that is, were elaborating a urine with more solute than solvent, thus demonstrating the presence of AVP. In those subjects whose urine data were collected ad lib there was a large change to positive free water clearance after the completion of the infusion. This large diuresis occurred concomitant with the observed fall in plasma AVP. The older subjects had a statistically significantly smaller diuresis which lasted a

a significantly briefer period consistent with the differences observed in the magnitude of and time course of the AVP response to ethanol.

Plasma osmolalities, corrected for ethanol content, rose initially from 290 mOsm to 295 mOsm, a level maintained to the completion of the study. This rise in osmolality may produce a positive stimulus for AVP secretion. Older subjects may respond to hyperosmolality despite the presence of ethanol which was not the response observed in younger individuals.

Analysis of response to 3% NaCl in 18 subjects divided equally into three age groups across the age spectrum awaits completion of the measurement of AVP by radioimmunoassay.

Significance to Bio-Medical Research and the Program of the Institute: There are clinical indications that the aged individual has a diminished ability to maintain salt and water homeostasis. Studies performed here in the past have examined the end organ phenomena that pertain to salt and water balance. Analysis of the central mechanisms involved is required to understand fully the impact of aging on the ability to maintain the internal milieu. Such understanding may alter clinical decisions about fluids and medications prescribed for the elderly patient.

Proposed Course of the Project: Completion of the hormone assays will be followed by a final compilation and synthesis of the data and its implications.

Keyword Descriptors: Antidiuretic hormone, arginine vasopressin, aging, ethanol, hyperosmolarity.

Honors and Awards: None.

Publications: None.

Serial No. Z01-AG-00006-02-CPB
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Metabolism Section
4. Baltimore, Maryland

PHSNIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Antipyrine metabolism in man: Influence of age, alcohol, caffeine, and cigarette smoking

Previous Serial Number: New Project

Principal Investigator: Robert E. Vestal

Other Investigators: Arthur H. Norris
Jordan D. Tobin
Bernice H. Cohen
Nathan W. Shock
Reubin Andres

Co-operating units: *Department of Epidemiology, School of Hygiene and Public Health, Johns Hopkins University

Man Years:

Total:	1.0
Professional:	0.8
Other:	0.2

Project Description:

Objectives: Aging has been previously reported to be associated with prolonged plasma half-life of antipyrine. Such age differences, however, could be due to other variables that both influence drug metabolism and differ with age. The purpose of this study was to examine antipyrine metabolism in participants in the Baltimore Longitudinal Study of Aging and to explore the possibility that age differences in such habits as alcohol use, caffeine consumption and cigarette smoking might influence drug metabolism in this population of healthy men.

Methods Employed: Plasma half-life (t 1/2), distribution volume, and metabolic clearance rate (MCR) were computed in 466 subjects aged 18 to 92. Of these 466 individuals, 307 had not taken medications and were considered suitable for analysis. Subjects received 1.0 gm antipyrine for the purpose of estimating total body water. Each subject was classified according to alcohol use, caffeine consumption and cigarette smoking. Chi-square and multiple regression analysis were utilized to determine significant associations of the variables and to assess

their relative importance in predicting antipyrine metabolism.

Major Findings: Half-life was 16.5 percent longer and metabolic clearance rate was 18.5 percent less in the old group than in the young ($p<0.025$). Regression equations for t 1/2 (hr) and MCR (ml/kg/hr) were t 1/2 = 10.7 + 0.06 (age) ($r = 0.15$, $p<0.01$) and MCR = 40.7 - 0.19 (age) ($r = 0.25$, $p<0.001$). Several additional significant associations ($p<0.05$ by χ^2) among the variables were identified: alcohol, caffeine, and cigarette use declined with age and were positively correlated with each other; MCR was directly related to caffeine and cigarette use, and the corresponding inverse relationship was found for t 1/2. Multiple regression analysis of MCR showed that when cigarette smoking and age were included, alcohol and caffeine, contributed little to the fraction of variance explained. MCR was predicted by the equation. MCR = 35.1 + 2.1(Smoking Class) - 0.1(Age) ($p<0.001$). Smoking accounted for 12% and age 3% of the variance in MCR.

Significance to Bio-Medical Research and the Program of the Institute: The significance of these results is that although age per se was associated with decreased antipyrine metabolism, exogenous factors (habits), which change with age and which also affect drug metabolism, may explain a large fraction of the overall age effect and must be taken into account in studies which attempt to quantify the effects of age on drug metabolism. Genetic variation undoubtedly accounts for much of the unexplained variance in metabolic clearance rate for antipyrine. Nonetheless, aging and factors such as smoking which are themselves influenced by age, play a demonstrable role in drug metabolism and may contribute to the higher incidence of adverse drug reactions in elderly patients.

Proposed Course of Project: The project is completed and a manuscript has been submitted for publication. The paper was selected for presentation at the Annual Meeting of the American Federation for Clinical Research.

Keyword Descriptors: Antipyrine metabolism, age, cigarette smoking, caffeine consumption.

Honors and Awards: None.

Publications: None.

Serial No. Z01-AG-00007-02-CPB
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1974

Project Title: The effects of age and ethanol on triglyceride lipase activities in postheparin plasma

Previous Serial Number: New Project

Principal Investigators: Robert E. Vestal
Jordan D. Tobin
Reubin Andres

Other Investigator: Ronald M. Krauss*

Cooperating Units: *Donnor Laboratory, University of California at Berkeley

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description

Objectives: Plasma levels of triglycerides (TG) increase, then decrease with advancing age. In addition plasma TG is known to increase following acute ethanol administration. The clearance of circulating lipoprotein triglyceride is thought to be mediated primarily by lipoprotein lipase. This enzyme activity is present in hepatic as well as a number of extrahepatic tissues and is released into the plasma after heparin administration. Post heparin lipolytic activity (PHLA) has been used to assess the activity of lipoprotein lipase. A method is now available to distinguish extrahepatic (protamine-inactivated or PI) from hepatic (protamine-resistant or PR) PHLA. The purpose of this project is to examine the effect of age on PHLA and the effect of acute ethanol administration on PHLA in an effort to increase our understanding of the mechanism by which plasma TG changes with age and under the influence of ethanol.

Methods Employed: Following routine diabetes testing, subjects in the Baltimore Longitudinal Study of Aging received an intravenous injection of heparin (10 units/kg). Blood samples were obtained for

plasma TG, cholesterol and PHLA. In addition some subjects who participated in the study of age and ethanol pharmacokinetics described in a separate report also received intravenous heparin in the same dose at the end of the 5-hour study period. Plasma TG and cholesterol were measured by standard methods and PR and PI fractions of PHLA were measured acccording to the method of Krauss and associates.

Major Findings: Preliminary analysis of PHLA data obtained following glucose testing in 50 subjects does not reveal significant correlations of either PR or PI with age, TG or cholesterol levels. Cholesterol and TG values were found to be 9 and 8 percent lower, on the average, after glucose testing when compared to the fasting values. The difference in cholesterol level was highly significant ($p<0.001$). The difference for TG was not significant. Of additional interest is the observation that mean values for PR (11.4 ± 0.65) and PI (3.3 ± 0.24) after glucose testing are significantly lower ($p<0.01$) than fasting values for PR (15.0 ± 0.85) and PI (4.2 ± 0.24) previously reported for a series of 40 male subjects.

In 38 subjects who received an injection of intravenous heparin 4 hours after intravenous ethanol administration similar differences in mean PR (11.0 ± 0.81) and mean PI (2.1 ± 0.21) were observed compared with the fasting control group. Once again there was no significant correlation of age with either PR or PI post-ethanol. TG are 19 percent higher ($p<0.05$) after acute ethanol. The change in TG was significantly correlated with age ($r= 0.37$, $p<0.025$) and with peak ethanol concentration ($r= 0.45$, $p<0.01$), but not with obesity index. This observation is confounded, however, by the observation that peak ethanol levels were also correlated with age ($r= 0.47$, $p<0.005$). Partial correlation analysis holding peak ethanol concentration constant showed that age was not correlated with the change in triglyceride.

Significance to Bio-Medical Research and the Program of the Institute: In essentially healthy males age has been shown to be unrelated to both PR and PI PHLA after both glucose testing and acute ethanol. This suggests that factors other than PHLA must account for the age differences in cholesterol and TG normally found.

Proposed Course of Project: The collection of data has been completed. Further data analysis and manuscript preparation is under way.

Keyword Descriptors: Age, ethanol, triglyceride lipase, heparin.

Honors and Awards: None.

Publications: None.

Serial No. Z01-AG-00008-03-CPB
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Cardiovascular Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Effect of age on the response to inotropic interventions and hypoxia of rat trabeculae carneae.

Previous Serial Number: HD-CP 17

Principal Investigators: Gary Gerstenblith
Harold A. Spurgeon

Other Investigators: Constantinos Limas*
Jeffrey Froehlich
Myron L. Weisfeldt**
Nathan W. Shock

Cooperating Units: *Department of Medicine,
Baltimore City Hospitals;
**Division of Cardiology, Department of Medicine,
Johns Hopkins University

Man Years:

Total:	1.75
Professional:	1.05
Other:	0.70

Project Description:

Objectives: The objectives are to characterize the effect of age on the physiology of cardiac muscle contraction and on the response of cardiac muscle to inotropic interventions. It had been demonstrated the previous year that contraction duration was prolonged in aged myocardium and that a prolonged active state resulting from delayed calcium removal from the contractile element may be responsible for this phenomenon. It had also been shown that age impairs the ability of cardiac muscle to mount an inotropic response to catecholamines. The present year's efforts were directed towards: (1) Examining the effect of age on the ability of the cardiac cell membrane to respond appropriately to an inotropic intervention. This was accomplished by examining the responsiveness to ouabain, a digitalis preparation. The physiology of the response was assessed by comparing the inotropic response to paired pacing with that to ouabain. The biochemical aspects of the response was determined by measuring the degree of ouabain binding to a membrane preparation and by measuring

the degree of inhibition of Na-K ATPase by ouabain, (2) Isolating the subcellular fraction thought to be responsible for calcium uptake in order to eventually determine if the age-related increase in contraction duration resulted from changes in this mechanism.

Methods Employed: (1) The performance of isometric trabeculae carneae was measured using techniques described in the 1972-1973 project report. For these experiments, however, the calcium concentration was lowered to 0.25mM. The extrasystolic potentiation was measured at coupling intervals from 400 msec to the mechanical refractory period. The response to ouabain was then measured at concentrations from 2x10-6M to 6x10-4M. The biochemical analyses were performed by Dr. Limas and the methods are described in his Guest Investigator Report. (2) The sacroplasmic reticulum was prepared according to the method of Harigaya and Schwartz (Cir. Res. 25:781, 1969).

Major Findings: (1) The sensitivity of aged cardiac muscle preparations to the inotropic and toxic effects of ouabain is decreased. The same inotropic stimulation can be achieved in the aged myocardium, but a higher concentration of the agent is required. However, the response to paired pacing is identical in both age groups. Baseline membrane Na-K ATPase activity is identical in young and old cardiac muscle. However, the binding of ouabain to the membrane preparation and the degreee of inhibition is decreased in aged myocardium. (2) Adequate preparations of cardiac sacroplasmic reticulum, capable of calcium uptake, can be isolated from young and aged rat myocardium.

Significance to Bio-medical Research and the Program of the Institute: (1) Aging significantly impairs the ability of the myocardium to respond to the digitalis preparation, ouabain. This is of interest since digitalis has a significant therapeutic role in the treatment of heart disease in the elderly as well as other age groups. These findings also localize this aging defect to the cardiac cell membrane. (2) The ability to isolate sarcoplasmic reticulum preparations in young and aged myocardium will enable the section to test the hypothesis that prolonged contraction duration is secondary to an aging change in the ability of the active relaxing system to remove calcium from the contractile element.

Proposed Course of the Project: (1) Identification of the mechanisms responsible for the age-associated decreased inotropic response to cathecholamines. (2) Examination of the effect of age on the calcium uptake of isolated sacroplasmic reticulum.

Keyword Descriptors: Rat heart, trabeculae carneae, aging, ouabain.

Honors and Awards: None.

Publications:

Lakatta, E. G., Gerstenblith, G., Angell, C. S., Shock, N. W., and Weisfeldt, M. L.: Prolonged contraction duration in aged myocardium. J. Clin. Invest. 55: 61-68, 1975.

Lakatta, E. G., Gerstenblith, G., Angell, C. S., Shock, N. W., and Weisfeldt, M. L. Diminished inotropic response of aged myocardium to catecholamines. Cir. Res. 36: 262-269, 1975.

Serial No. Z01-AG-00009-01-CPB
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Cardiovascular Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Dynamic mechanical properties of aged myocardium

Previous Serial Number: New Project

Principal Investigators: Harold A. Spurgeon
Phillip T. Thorne

Other Investigators: Gary Gerstenblith
Myron Weisfeldt*
Nathan W. Shock

Cooperating Units: Baltimore City Hospitals
*Division of Cardiology
Johns Hopkins University

Man Years:

Total	1.4
Professional:	1.2
Other:	0.2

Project Description:

Objectives: The purpose of this project is two-fold. First, the basic cardiac muscle model as it applies to isolated, isometric rat myocardium is to be extended and quantified in order to define the role and relative importance of each of the elements comprising the normal adult myocardium. Concurrently, the age-associated changes in each of these structural elements are to be defined. The second element involves the role of the dynamic mechanical properties in the transduction of the metabolic process of contraction into externally manifest work. Here, we are concerned not only with the effect of an exogenous agent on the metabolic machinery, but also with its effect on the inherent stiffness-determining properties of the muscle. It is possible that the two sites (metabolic and structural) may be affected diametrically by a given agent studied as a function of age; this could account for the relatively unimpressive age changes observed to date in isolated myocardial preparations.

Methods Employed: Isolated superfused trabeculae carneae obtained from adult and senescent rats produced by Gerontology Research Center Aging Colony are used as study material. These muscles are clamped in an isometric configuration in such a manner that known controlled length changes may be superimposed on the isometric state. A typical sine wave forcing function can be applied in the present apparatus at frequencies less than 0.001 Hz to over 80 Hz. These length changes range from 0.5 mm down to the system noise at 0.0002 mm. Measurement of the phase and amplitude relations of the resulting force produced by the length change allows calculation of the viscous and elastic components of the muscle. The muscle is stimulated synchronously, to produce a contraction. Thus time-dependent effects of the individual elements can be studied during the contractile response.

Major Findings: The primary research effort of this new program has been in development of apparatus and mathematical models for accurately describing the magnitude and time course associated with dynamic changes in the mechanical components of heart muscle. A substantial portion of this total effort has been directed toward testing the many assumptions inherent in accepted cardiac models for their applicability and accuracy in the rigorous treatment necessitated here. Numerous revisions were found necessary in both the basic apparatus and computational approach to provide solutions for some of these problems. Application to aging myocardium has been begun. Early results show that an apparent decrease in the internal series spring is associated with advancing age.

Significance to Bio-medical Research and the Program of the Institute: Accurate data regarding the mechanical structures responsible for the transmission of metabolically generated force to manifest external length or force changes is essential to our understanding of the age-related differences in myocardial performance. Both the magnitude and time course of force transmission are profoundly affected by changes in the dynamic response characteristics of the "springs and dashpot" inherent in living tissue. The extent to which modification in these connective elements occurs as a result of changes in cellular environment and specifically due to influence of inotropic agents may change the interpretation of the mechanism of action of these important regulatory agents. Age dependent effects at any step of the chain could have profound implications in interpretation of cardiac performance data.

Proposed Course of the Project: (1) complete and extended current model; (2) calculate internal length changes occurring in "isometric state"; (3) investigate role of Ca++ and catechols and other inotropes, specifically looking for age-dependent changes in effects on mechanical elements; (4) determine the role of receptor-mediated agents on spring constants independent of metabolic effect; (5) study electromechanical coupling and extrasystolic potentiation, to determine differential effects with age.

Keyword Descriptors: Aging, rat heart, trabeculae carneae, myocardial contraction.

Honors and Awards: None.

Publications: None.

Serial No. Z01-AG-00010-02-CPB
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Cardiovascular Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Hemodynamics of the left ventricle and mitral valve in aging man

Previous Serial Number: HD-CP 31

Principal Investigator: Gary Gerstenblith

Other Investigators: James Frederiksen*
Nicholas J. Fortunin*
Myron Weisfeldt*
Nathan W. Shock

Cooperating Units: Baltimore City Hospitals
*Division of Cardiology
Johns Hopkins University

Man Years:

Total:	1.15
Professional:	.75
Other	.40

Project Description:

Objectives: To determine the effect of age, in man, on the structure and function of the left ventricle, the aorta, and the mitral valve.

Methods Employed: The mitral valve, posterior left ventricular and septal wall motions will be recorded by echocardiography while the subject is in the supine position at rest and during blood pressure increase induced by hand grip exercise. Simultaneous EKG, carotid pulse, apex cardiogram, and phonocardiogram traces will also be obtained. Posterior left ventricular wall thickness will be read directly from the record. The change in minor axis dimension between the septum and posterior wall in systole and diastole will be utilized to determine left ventricular volume, ejection fraction, and velocity of circumferential fiber shortening. The intervals between the QRS complex and mitral valve closure, and between the carotid pulse

incisura and mitral valve opening will provide indices of electro-mechanical delay, isometric contraction, and isometric relaxation respectively. The mean rate of mitral valve opening is obtained by computing the slope between the E and F points on the mitral valve motion.

Significance to Bio-medical Research and the Program of the Institute: In the absence of mitral valve disease, the E-F slope of the mitral valve is thought to reflect largely compliance characteristics of the left ventricle during early diastole. The decreased slope with increased age indicates decreased compliance and suggests that one of the major age changes in human myocardium is an impaired relaxing ability. Such a change would not appear to alter performance under basal conditions but would adversely affect the cardiac adjustment to rapid heart rates under conditions of such stresses as exercise or certain disease states. Increased aortic root diameter would increase cardiac workload by increasing the inertial forces which must be overcome by the left ventricle in early systole. Changes in stroke volume with age would not necessarily indicate intrinsic myocardial alterations because the results would have to be correlated with any possible age changes in preload.

Major Findings: Analysis of results from the 38 participants in the Baltimore Longitudinal Study of Aging has been carried out. Subjects cover the entire adult age span. These studies are of course cross-sectional only at this stage of the study. In the older subjects two significant differences were found: (1) The E-F slope of the mitral valve was lower and (2) systolic and diastolic aortic diameters were greater. In addition stroke volume was lower, but this was not statistically significant at this time.

Proposed Course of the Project: Continuation of the studies in the resting state to establish age associated norms for echocardiographic parameters related to the heart. A possible age differential in the effect of stress on these parameters will also be examined. These studies will extend the information obtained from animal models to intact man.

Keyword Descriptors: Aging, echocardiography, left ventricular function, mitral valve function.

Honors and Awards: None.

Publications: None.

Serial No. Z01-AG-00011-03-CPB
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Endocrinology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Hormones, hormone receptors, and aging. I. Effects of age on hormone-mediated alterations of adenylate cyclase, tissue cyclic AMP and factors controlling these metabolic regulators.

Previous Serial Number: HD-CP-18

Principal Investigators: Robert I. Gregerman
Barry Cooper
Michael Katz (E.O.D. July 1, 1974)

Other Investigators: None

Cooperating Units: Department of Surgery
Baltimore City Hospitals

Man Years:
Total:
Professional:
Other:

Project Description:

Objectives: The initial event in the mechanism of action of a variety of hormones is the activation of cell surface adenylate cyclase. the enzyme responsible for the production of cyclic adenosine-3'-5' monophosphate (c-AMP). Numerous cell functions appear to be under control of this key substance. Although aging is known to be accompanied by a variety of altered responses to hormones, the role of adenylate cyclase in these events has been barely explored. Our investigations are designed to provide new information in this area of hormone action in an attempt to understand the mechanisms by which aging affects hormone responsiveness (sensitivity). Since adenylate cyclase is a membrane-bound enzyme, such studies may also give insights into age-related alterations of cell membranes.

Methods Employed: Adenylate cyclase is determined by a labeled substrate assay in which ^{32}P-ATP is converted to c-AMP by action of the enzyme. ^{14}c-AMP is isolated by a double-column technique (Salamon, et al). Use of ^{14}C and ^{3}H c-AMP allow precise quantitation of the recovery in each experiment. Assays are performed on tissue homogenates, particulates, and purified cell membrane fractions isolated by standard methods of gradient centrifugation.

For studies of fat cells, tissue samples are digested with collagenase to yield isolated cells prior to preparation of cell membranes. The cells are counted and sized (after fixation in OsO_4) in a Coulter apparatus.

Major Findings: 1. Adenylate Cyclase of fat cells of the rat during growth and aging: The enzyme has been quantitated in cells isolated from epididymal fat pads of animals 1, 2, 6, 12 and 24 months. Activity has been expressed per mg membrane protein and per cell. Basal and activated enzyme has been studied for a catecholamine and two polypeptide hormones (epinephrine; glucagon and ACTH), by the anion activator, fluoride, and by the nucleotide activator, 5'-guanylyl-imidodiphosphate (Gpp(NH)p). The results show that growth and age-dependent changes follow different patterns for each hormone studied.

a) Basal activity: Activity per cell increased 3-fold between 1 and 2 months (as cell surface area increased 2.1 fold) and then decreased.

b) Epinephrine: Activity per mg protein was stimulated 8-fold over basal at all ages but declined by 60% between 2 and 24 months. The ratio stimulated:basal activity remained constant, however.

c) Glucagon: Activity per mg protein and per cell declined rapidly from peak levels at 1-2 months of age so that by 6 months hardly any hormone responsiveness remained. The enzyme of the adult animal (12, 24 months) is totally unresponsive to this hormone.

d) ACTH: Stimulated activity was maximal (4.5 fold) at 1 month and declined slowly thereafter, but was still twice basal at 24 months. The ratio of stimulated:basal activity declined unlike that for epinephrine.

e) Fluoride activation: The pattern seen was very similar to that for epinephrine, suggesting a gradual loss of the catalytic portion of the receptor-transducer-catalytic cyclase complex.

f) Gpp(NH)p activation: This nucleotide activates cyclase very strongly, especially in the presence of a high concentration of Mg^{++} ion. The pattern of activation is similar to that for fluoride. Since Mg^{++}-Gpp(NH)p is thought to activate "maximally", the evidence suggests that aging in the adult portion of the rat's life-span is accompanied by loss of catalytic enzyme from the adipocyte membrane. Other evidence suggests that the dramatic loss of glucagon responsiveness is due to loss of cell surface hormone receptors. No such explanation need be invoked for epinephrine-sensitive hormone.

g) Relation of cell size to cyclase activity: Heavy emphasis has been placed by others on the influence of cell size, per se, on hormone responsiveness, especially for glucagon and insulin. Our observations show that cyclase activity per cell increases as cells and their membranes grow, but the decreased enzyme during aging is not due to changing cell size, since no change occurs after 6 months, while cyclase decreases.

2. Relation of the loss of glucagon responsiveness to cell size. Others have suggested that increasing cell size is responsible for loss of glucagon responsiveness (lipolytic). However, we have now subjected 1 year old rats, which have no glucagon responsive cyclase, to caloric restriction. Weight loss was accompanied by a reduction of cell size to that of younger, hormone-responsive animals. However, no return of glucagon sensitivity occurred.

Increasing cell size, therefore, is not likely to be responsible for the observed loss of glucagon response. Furthermore, caloric restriction of growing animals prevented fat cells from increasing in size, but did not prevent an age-related loss of cyclase activity. Caloric restriction increased cyclase activity.

3. Characterization of adenylate cyclase from human fat cells: No information has previously been published for the human enzyme. Because of possible species differences implied by differences of lipolytic responsiveness of fat from man and rat, we have undertaken characterization of the human enzyme as a preliminary step to studying the relationship of aging to cyclase in human material.

4. Anion activation of adenylate cyclase: Further studies have been performed to expand our finding last year that anions other than fluoride activate cyclase. We have now defined the time course of activation of hepatic cyclase by chloride ion in comparison with fluoride. These results show that chloride, at early times after addition (up to 3 minutes) activates more strongly than fluoride while the latter shows an accelerating pattern. A number of other differences have been defined; activation by azide ion has been studied, as has the instability of chloride-activated enzyme and its relationship to the epinephrine-stimulated enzyme. Comparison of anion activation of enzyme in homogenates and from purified membranes is underway.

Significance to Bio-medical Research and the Program of the Institute: These studies are beginning to define one of the potentially most important areas of aging and hormone action, the adenylate cyclase-cyclic AMP "second messenger" system. We have already established in aging animals that the patterns and mechanisms of age effects vary with the tissue involved (liver vs fat) and are dependent on the particular hormones involved; that species differences of cyclase responsiveness (e.g., between rat and man) occur even for the same tissue (fat); and that nucleotide-activator effects may be related to the apparent species differences. The studies offer an approach to age-related changes of membrane function.

Proposed Course of the Project: Further attempts will be made to define the mechanism of the age-influenced increase of epinephrine-sensitive adenylate cyclase in the liver. Studies on human adipose adenylate cyclase will continue and a cross-sectional aging study initiated. Further studies comparing cyclase in minimally altered membranes (homogenates) with those obtained by extensive purification will be continued.

Keyword Descriptors: Aging, hormones, cyclic AMP, adenylate cyclase, fat cells, glucagon, epinephrine, ACTH.

Honors and Awards: Dr. Robert I. Gregerman was invited to be a visiting scientist at the Weizmann Institute of Science, Rehovot, Israel, June, 1975.

Publications: Kalish, M. I., Pineyro. M. A., Cooper, B. and Gregerman, R. I. Adenylyl Cyclase Activation by Halide Anions Other than Fluoride, Biochemical and Biophysical Research Communications 61, 731-737, 1974.

Serial No. Z01-AG-00012-03-CPB
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Endocrinology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Hormones, hormone receptors and aging. II. Effects of age on hormone binding and responsiveness in target cells and tissues.

Previous Serial Number: HD-CCP-18

Principal Investigator: George S. Roth

Other Investigator: Robert I. Gregerman

Cooperating Units: None

Man Years:
Total:
Professional:
Other:

Project Description:

Objectives: Aging organisms exhibit an altered ability to respond to hormones. Data from this and other laboratories suggest that at least some age-related changes in biochemical responsiveness may be due to alterations in hormone binding by intracellular receptors (steroid hormones) and surface receptors (catecholamines, polypeptides) of target cells. Present efforts are directed toward 1) determination of whether changes in hormone binding are a generalized cause of altered responsiveness during aging, and 2) elucidation of the cellular and molecular mechanisms responsible for such changes.

Methods Employed: Hormone responsive cells are isolated, purified and fractionated from whole tissues. Anti-sera are prepared to defined cell populations. Binding assays are performed using labeled hormones (glucocorticoids; catecholamines; specific catecholamine antagonists). Lipolysis, nutrient transport, cell morphology and cell viability studies are performed by standardized techniques.

Major Findings: Previous studies in this laboratory demonstrated that decreased glucocorticoid inhibition of uridine uptake into splenic leukocytes of senescent rats is due primarily to decreased concentrations of intracellular receptors for these hormones. Data obtained during this past year show that cell yield per unit spleen weight and protein content per cell are comparable in mature (12-14 mo.) and senescent (24-26 mo.) rats. In addition, splenic cell populations are composed of 95-98% morphologically identifiable lymphocytes in both age groups. Glucocorticoid responsive cells appear to be randomly distributed among thymus derived and non-thymus derived splenic lymphocytes. It is there-

fore difficult to determine whether receptors are lost from target cell or target cells are lost from the splenic populations, until such time as specific isolation procedures for glucocorticoid-sensitive cells are available.

Many other studies on hormone binding and responsiveness during aging have also employed systems which contain heterogeneous cell types that undergo proliferation and/or turnover during the lifespan. In these systems, alterations in hormone binding may thus relect age-related variations in the proportion of one cell type to another, molecular changes intrinsic to one or more cell types, or both of these possibilities. In addition, different cells in such target tissues may be of different ages. Consequently, it is of value to examine hormone binding and responsiveness in defined populations of post-mitotic cells from animals of different ages.

Previous studies in this laboratory showed reduced concentrations of glucocorticoid binders in brain cerebral hemispheres and epididymal fat pads (among other tissues) during aging. In order to overcome the problems involved in studying complex tissues as mentioned above, defined populations of fixed post-mitotic cells (neurons and adipocytes) were examined during this past year.

Neuron cell bodies were isolated from cerebral cortices of mature and senescent rats by mechanical disruption and density gradient sedimentation. Preparations contain essentially all neurons as judged by morphology. Moreover, the degree of neuronal purity judged by biochemical markers, as well as yield, physical integrity and protein content per cell are comparable in both age groups. Glucocorticoid binders are reduced in concentration by 60-65% in old (24-26 mo.) neurons. Exposure of isolated neurons to glucocorticoids for 1-3 hours in culture, followed by administration of ^{3}H-norepinephrine causes a 25-35% stimulation of ^{3}H uptake in the mature (12-13 mo.) group. This may represent a control mechanism in the neurotransmission process, since catecholamines serve as neurotransmitters. The radioactivity taken up is more than 75% acid soluble and cell to media concentration ratios approach 10. Preliminary experiments suggest that glucocorticoid-stimulated ^{3}H-norepinephrine uptake is reduced to less than 10% in cells of old rats. Thus, age changes in neuronal glucocorticoid binding and responsiveness may be closely related.

In another study of hormone binding and responsiveness in defined post-mitotic cells during the past year, adipocytes were prepared from epididymal fat pads. Such cells have been shown to be of equal size and protein content in mature (12-13 mo.) and senescent (24-26 mo.) rats by several investigators. Preliminary findings in this laboratory show a 50-60% reduction in glucocorticoid binder concentration in adipocytes from senescent rats. Glucocorticoid stimulated lipolysis in these same cells appears to be reduced by at least 60%, although basal levels are not significantly different in mature and old cells. As with splenic leukocyte and cortical neurons, therefore, age related reduction in glucocorticoid binder concentration correlates well with decreased biochemical responsiveness.

Significance to Biomedical Research and the Program of the Institute: Altered ability to respond to hormones is an important manifestation of the generalized decrease in vitality which is characteristic of senescence. The present study seeks to elucidate the cellular and molecular mechanisms by which such generalized changes in responsiveness occur. Application of current knowledge in cell biology and molecular endocrinology to gerontological problems will yield data of value to all three fields. Studies of age-changes in hormone receptors

are of particular interest now that methods for regulating receptor quantity and quality are becoming available. Such manipulations offer hope of possible reversal of age-related impairments in responsiveness by reconstitutions of altered hormone receptor systems.

Proposed Course of the Project: Additional studies will attempt to establish whether definitive and causal relationships exist between age changes in hormone binding and responsiveness in the systems mentioned above. It is hoped eventually to obtain human tissue for comparison with age changes in the rat. Other members of the Endocrinology Section are already engaged in such comparisons, and use of their protocols should facilitate expansion of the present study. Ultimately, we hope to elucidate the cellular and molecular mechanisms responsible for changes in hormone binding and responsiveness during aging. This may entail purification of hormone receptors, nutritional and other environmental manipulations of experimental animals, and investigations into the regulation of receptor quantity and quality.

Keyword Descriptors: Aging, hormones, receptors, hormone-responsiveness.

Honors and Awards:
Dr. Roth was an invited partcipant to the NICHD "Pathobiology of Aging Course" at Cornell University, August, 1974.

Dr. Roth was an invited lecturer at the "Gordon Research Conference of the Biology of Aging" at Tilton, N.H., August, 1974.

Dr. Roth was an invited lecturer at the "Symposium on Endocrine Regulatory Mechanisms", 10th Intl. Congress of Gerontology, Jerusalem, Israel, June, 1975.

Dr. Roth was elected to Fellowship in the Gerontological Society, Nov., 1974.

Dr. Roth was elected chairman of the Membership-Fellowship Committee of the Biological Sciences Section of the Gerontological Society for 1975-76.

Publications:
Roth, G. S., Age-related changes in glucocorticoid binding by rat splenic leukocytes: possible cause of altered adaptive responsiveness. Federation Proceedings 34: 183-185, 1975.

Roth, G. S. and Adelman, R. C., Age related changes in hormone binding by target cells and tissues; possible role in altered adaptive responsiveness. Experimental Gerontology, 10 1-11, 1975.

Roth, G. S., Changes in hormone binding and responsiveness in target cells and tissues during aging. Proceeding of the Philadelphia Symposium on Aging, Plenum Press, New York (in press).

Roth, G. S., Altered hormone binding and responsiveness during aging. Proceedings of the 10th International Congress of Gerontology (in press).

Roth, G. S., Reduced glucocorticoid responsiveness and receptor concentration in splenic leukocytes of senescent rats. Biochimica et Biophysica Acta (in press).

Serial No. Z01-AG-00013-01-CPB
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Endocrinology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 thru June 30, 1975

Project Title: Hormones, hormone receptors, and aging.
III. Effects of age on the human male reproductive system.

Previous number: none

Principle Investigators: S. Mitchell Harman (E.O.D) July 1, 1974)
C. Martin

Other Investigator: R. I. Gregerman

Man years:
Total:
Professional:
Other:

Project Description:

Objectives:

A. Background- The reproductive system of men and women works under a complex series of controls which determine the orderly sequence of function from embryonic development, through puberty, the period of mature function, and finally senescence. The hypothalamus (an area at the base of the brain) modulates pituitary function by secreting releasing hormones into vessels which drain directly into the pituitary (pituitary portal system). One of these hormones, LHRH, controls pituitary synthesis and release of the gonadotrophins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). The gonadotrophins in turn control function of the gonads. In the male, LH acts on the Leydig cells of the testes to stimulate secretion of testosterone, the primary male hormone, while FSH and testosterone together influence the seminiferous tubules of the testes to produce mature sperm. In addition, testosterone produces and maintains masculinization of the body (beard growth, increased body hair, increased lean muscle mass), male libido, and, importantly, "feeds back" on the hypothalamus and pituitary to regulate the secretion of gonadotrophins.

B. Current knowledge- While much is known concerning the events of embryonic development, puberty, and mature function of the reproductive system, the nature of the physiologic events surrounding senescence of this system are not yet clear. The best recent publications in this area are studies

by Vermeulen and Verdonck, by Rubens, Dhont, and Vermeulen, and by Stearns et al. These workers agree that there is substantial loss of Leydig cell function beginning in men in their 50's who demonstrate a decrease in circulating "free" (non-protein bound) testosterone and an increase in LH and FSH. Whether there is simultaneous loss of pituitary or hypothalamic function is not clear, although the response of elderly men to LHRH injections is reported to be normal. In addition Stearns et al noted decreased testicular volume and decreased facial, pubic, and axillary hair in their older subjects. Neither group, however, attempted to correlate individual testosterone levels with changes in secondary sex characteristics, libido, onset of prostatism, or development of cardiovascular disease (long known to be more frequent in males), nor were there studies of seminiferous tubular function in terms of quantity and quality of sperm production. Finally, all three studies suffer from the limitation of being cross-sectional rather than longitudinal, so that if reproductive system variables (such as characteristic plasma testosterone) influence longevity, the population studied might become progressively skewed with advancing age.

C. Proposed study- Objectives of this study are 1) to provide normative information on reproductive function in the aging human male with regard to semen production, gonadotrophin secretion and pituitary gonadotrophin reserve, and androgen production and testicular (androgen) responsiveness to gonadotrophins; (2) to elucidate the relationships of pituitary, Leydig cell, and seminiferous tubular function in the aging male, with the object of determining the site (or sites) of failure of the reproductive system; and (3) to correlate behavioral and health variables such as libido and prostate disease with levels of endocrine function in the aging human male.

Methods employed: (1) Gonadotrophins will be assayed using a double antibody radioimmmunoassay adapted from that employed by Rayford and Ross. (2) Testosterone and dihydrotestosterone will be measured using a charcoal type radiommunoassay adapted from that described by Nieschlag and Loriaux. (3) Assay results will be analyzed by the computerized method of Rodbard. (4) Semen analysis will be done using standard techniques for determining number and quality of sperm. (5) Several methods are under active consideration for determining serum protein binding of testosterone and hence the free testosterone fraction. (6) Clinical samples will be obtained in two ways: (a) Longitudinal Study subjects will be given endocrine provocative tests with multiple serum samples taken at intervals. This will provide both baseline measurements and data regarding ability of the system to respond when stimulated. These tests are the LHRH stimulation test for pituitary gonadrotophin reserve, and the HCG stimulation test for Leydig cell reserve. In addition a series of semen samples will be collected from those subjects able to cooperate and libido will be estimated from a questionaire prepared by Dr. Martin. (b) Freeze dried plasma samples, taken from longitudinal subjects in past years, will be analyzed in order to compare gonadotrophin and androgen levels with libido scores previously obtained by Dr. Martin and with other health variables.

Progress to Date: (1) Radioimmunassays for both FSH and LH have been developed and shown to produce satisfactory standard curves using automated pipetting apparatus in our lab. A new method utilizing concavalin A affinity chromatography has been adopted to improve purity of FSH and LH tracer material. (2) A radioimmunoassay for testosterone is in the final stages of development using a gas driven celite column system to separate component steroids in plasma extracts and using an automated sampling method to separate bound from free hormone.

Significance to Biomedical Research and the Program of the Institute: Little or no information has been gathered on the function of the reproductive system in aging, despite growing interest, on the part of society and medicine, in sexual function in aging people and to its relationship to general health. This project is designed to study aging of a body system which has heretofore not been investigated here, and will therefore serve to enlarge the GRC data base on aging processes obtained by the Longitudinal Study. In addition, information obtained will serve as a basis for the construction of hypotheses regarding the nature of the aging process in endocrine systems. Such hypotheses will then be open to investigation in animal models, in which experimental investigations may in turn provide insights into the general mechanisms of aging.

Proposed Course of Project: To date all efforts have been directed at developing standard methodology for measuring the hormones in question. This phase is nearly complete and the next phase will be to begin collecting samples from clinical subjects according to the protocol devised.

Keyword Descriptors : Aging, hormone, testosterone, gonadotrophins, FSH, LH.

Publications: None

Honors and Awards: None

Serial No. Z01-AG-00014-05-CPB
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Endocrinology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The biochemistry of renin.

Previous Serial Number: HD-AG 21 (C)

Principal Investigators: Robert I. Gregerman
Hardy J. Chou

Man Years:
Total:
Professional:
Other:

Project Description:

Objectives: Renin is a proteinase which is secreted by the juxtaglomerular cells of the kidney. A variety of factors control renin release. In the circulation renin acts on a plasma glycoprotein to release angiotensin I, a decapeptide which is activated by conversion to a smaller octapeptide, angiotensin II, in the lung. Circulating angiotensin II influences the secretion of aldosterone, principal mineralocorticoid of the adrenal, and has other effects on the cardiovascular system. In certain pathologic states renin has a direct role in the pathogenesis of disease, as it does in renovascular hypertension. Renin is also implicated in other forms of hypertension. The secretion of renin (and aldosterone) is markedly influenced by aging in man, and hypertension is an age-dependent disease.

Because of these considerations, our laboratory has had an interest in renin and angiotensin, especially in development of new techniques for measurement of the enzyme and its peptide products, in the biochemistry of the enzyme and its substrate, and the relevance of the renin-angiotensin-aldosterone system to normal and pathologic aging.

Methods Employed: Renin has been assayed by our previously published polymeric substrate assay. Renin substrate has been partially purified from porcine plasma. Other techniques are standard analytical procedures of peptide chemistry (amino acid analysis, high voltage electrophoresis, etc.).

Major Findings: I. Studies of pepstatin inhibition of human renin we reported last year have been completed. New kinetic data indicate that pepstatin is an extremely potent, non-competitive inhibitor of the human enzyme ($K_I = 10^{-10}$ M). The kinetic data are virtually the same as those for pure pepsin in the same assay system. This information, taken together with our previous demonstration that renin is inhibited by a diazoacyl reagent, establishes a close relationhip of renin to other so-called acid proteinases (e.g. pepsin, cathepsin D). All of these enzymes have aspartic acid at their active centers.

II. The Chemical Nature of Renin's Natural Substrate: Renin appears to possess unparalleled and unique specificity among proteinases. However, this apparent specificity may be related to the chemistry of the natural glycoprotein substrate rather than to the proteinase. We have attempted to explore this possibility with the hope of eventually being able to synthesize a labeled renin substrate which will prove practical for rapid assay of renin in human plasma.

We have partially purified porcine glycoprotein substrate. In confirmation of a report by others, treatment with alkali under specified conditions yields a product which appears to be the tetradecapeptide N-terminal fragment. This finding suggests that the C-terminal serine of the tetradecapeptide is involved in a unique linkage to the protein, viz., an ester bond. However, we have now secured evidence that certain seryl peptide bonds are unusually susceptible to alkaline cleavage. Thus we have used more specifc esterolytic conditions (hydroxylamine and hydrazine at pH 10). Despite the fact that the same product is again obtained, the general stability of alkali sensitive seryl peptides is under close study, and a variety of experiments are underway to determine more precisely the nature of the attachment of this N-terminal fragment to the glycoprotein substrate.

III. Polymeric inhibitors of renin: Methods have been devised to link pepstatin to certain polymers. These high molecular weight polymers should have long biological half-lives and may prove to be useful in vivo. Characteristics of the pepstatin-polymer complex have not yet been explored.

Significance to Bio-medical Research and the Program of the Institute: Our studies have defined the chemical relationship of renin to other proteinases. Present studies may allow an explanation of renin's specificity and the development of new classes of renin inhibitors and synthetic renin substrates. This information may be useful eventually for practical applications to problems related to the diagnosis and treatment of hypertensive diseases.

Proposed Course of the Project: Our immdiate objective is an elucidation of the nature of the chemical bond between the N-terminal fragment of renin's substrate and the remainder of the glycoprotein substrate. Testing of the pepstatin-polymeric substrate will be undertaken.

Keyword Descriptors: Renin, renin substrate, peptides, hypertension.

Honors and Awards:

Dr. Robert I. Gregerman was an invited speaker at a Symposium honoring the renowned endocrinologist, Jerome W. Conn, at the University of Michigan, in Ann Arbor, Michigan, Oct. 18, 1974. Dr. Gregerman spoke on the topic "Renin: Biochemical Relationship to Other Proteinases."

Publications:

Workman, R. J., McKown, M. M., and Gregerman, R. I., Renin: Inhibition by Proteins and Peptides. Biochemistry 13, 3029-3035, 1974.

McKown, M. M., Workman, R. J., and Gregerman, R. I., Pepstatin Inhibition of Human Renin. Kinetic Studies and Estimation of Enzyme Purity. Journal of Biological Chemistry 249, 770-774, 1974.

McKown, M. M., and Gregerman, R. I., Human Renin Inhibition by a Diazoacyl Reagent: Relationship of the Enzyme to Other Proteinases. Life Sciences 16, 71-79, 1975.

Serial No.: Z01-AG-00015-17-CPB
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Longitudinal Studies of Human Physiology, Biochemistry and Psychology

Previous Serial Number: HD-CP-23

Principal Investigators: N. W. Shock, Director
Reubin Andres, Clinical Director
Arthur H. Norris, Coordinator

Other Investigators:

D. Arenberg	C. E. Martin
B. D. Bricker	C. C. Plato
M. Butler	J. D. Tobin
B. T. Engel	S. P. Tzankoff

Cooperating Units: Baltimore City Hospitals

Dr. Bernice H. Cohen
School of Hygiene & Public Health
The Johns Hopkins University

Dr. James Schlesselman
Biometry Branch, NICHD-NIH, Bethesda

Man Years:

Total:	11.15
Professional:	3.15
Other:	8.00

Project Description:

Objectives: This project is designed to: (1) secure replicate measures of physiological, pathological, biochemical and psychological variables on longitudinal study participants at specified intervals; (2) summarize and compare the results of testing in relation to age according to cross-sectional and longitudinal formats; (3) identify characteristics of individual participants which may be related to changes of function over time and to age at death; and, (4) determine whether the data obtained support one or another theory of the mechanisms responsible for age-related functional decrements.

Methods Employed: The Sample: Study participants are male volunteers recruited by other participants in the program. Recruits agree to return to GRC in Baltimore for 2-1/2 days of testing every 12 months (age 70 and over), 18 months (age 60-69) or 24 months (under age 60) for an indeterminate period. At entry into the program, 87% of subjects reported at least some college, 87% were identified with professional, technical or managerial occupations, 90% were presently married, 81% described themselves as financially comfortable, and of the group who returned for the fifth visit, 90% had rated their health as good or excellent on both first and fifth visits.

Testing Procedures: Psychological tests include especially designed procedures which assess verbal learning and problem-solving abilities as well as standardized tests of intelligence and personality. Physical examinations and medical histories are performed with check lists to insure completeness. Smoking habits, dietary intake, and marital and sexual adjustments are obtained by questionnaire, diary and interview. Both conventional and special procedures are used to assess physiological areas such as kidney function, pulmonary function, neuromuscular function, cardiovascular function, and special senses.

Data Management: Medical records and test results are maintained in written form in the laboratory and transferred to a data retreival and analysis system by keypunching on tabulation cards or by recording the test results directly on punched paper tape or magnetic tape. Data are maintained and used in ways which protect the privacy of participants. Sensitive material is specially encoded. Individual scientists review, evaluate and summarize the data for scientific reporting.

Major Findings: As of December 31, 1974, 1012 subjects had been tested over one or more visits to GRC, accounting for a total of 5051 participant-visits since 1958. By this date, 615 subjects had completed four or more visits while 238 had been tested over eight or more visits. Since the study's inception, 135 subjects have died, 86 have formally withdrawn, and 155 failed to respond to inquiry, leaving a current active sample of 636 participants.

The development of an automated metabolism analyzer has made possible repeat testing of responses to arm exercise in subjects of various ages. The initial tests were performed in the early 1960's for longitudinal studies participants and in the early 1950's for staff members who are available for retesting. Preliminary results show higher responses for oxygen uptake, carbon dioxide production and ventilation volume in the current tests compared with the tests performed ten or more years earlier. The comparison also shows a slower recovery of ventilation and carbon dioxide production in the current tests. These changes may reflect reduced mechanical efficiency of muscle contraction and increased pulmonary dead space with incresing age. This automated metabolism analyzer has a variety of uses. Another use will be to quantify the metabolic responses to exercise performed on the treadmill during the exercise electrocardiogram test.

The changes in learning and problem solving and in the information capacity of a hand-eye coordination feedback loop in subjects over 65 years of age again focusses on the traditional age of retirement. Findings of age differences related to age 65 have been previously reported for motor functions related to central nervous system competence, such as mechanical efficiency of arm exercise and muscle strength. All of these variables showed little or no difference or change between 30 and 64 years of age and either changes or differences in the direction of poorer CNS function for subjects over age 64. While no mechanism has been devised to relate these findings, the onset of decline in CNS function in the average subject is established at age 65. It is of interest to consider what leads to retirement. Many reasons have been considered, such as company rules, social pressure and health. We have asked if there is any functional basis for these customs and practices. The question is difficult to answer since the role of functional change is not clear. Functional change could have led to the establishment of the social milieu relative to work. On the other hand, the social situation of an individual might lead to a decline in function in the sense that he no longer participates because he is not expected to. There are many anecdotes about individuals who have refused to give in to social pressures and have maintained youthful behaviors into later life. The ability to maintain motor function and strength is particularly impressive. Many participants in the longitudinal studies increase their activity levels at retirement. This attempt to return to the patterns of youth is not often sustained. It does, however, reflect a desire to postpone the aging so clearly implied by retirement from a life's career.

Significance to Bio-Medical Research and the Program of the Institute: A major goal of the longitudinal Program is a deeper understanding of age-related changes in the different organ systems, and their interrelationships. The relation of functional changes in an individual to age at death, age of onset of a disease, and other end points is important for understanding aging in humans and the impact of aging on society. The intensive study of multiple variables will also provide tests of risk-factor theories for specific age-related diseases.

Proposed Course: Data collection and analyses will be continued. Continued emphasis on automation of tests, data entry, and analyses should provide improved accuracy and efficiency. A major summary of all aspects of this program is in progress.

Keyword Descriptors: Age, Longitudinal Studies, Physiology, Psychology

Honors and Awards: None

Publications: None

Serial No. Z01-AG-00016-20-CPB
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Age Changes in Human Performance

Previous Serial Number: HD-CP-24

Principal Investigators: Arthur H. Norris
James E. Fish
Fred Garfinkel

Other Investigators:

N. W. Shock	Harold Menkes**
Stephen P. Tzankoff	Richard Rosenthal**
A. T. Welford*	Warren Summer**
Solbert Permutt**	Philip Norman**
Douglas G. Carroll***	

Cooperating Units: Baltimore City Hospitals***

Department of Psychology*
University of Adelaide
Adelaide, South Australia

The Johns Hopkins Medical Institutions**
Baltimore, Maryland

Man Years:

Total:	5.75
Professional:	2.75
Other:	3.00

Project Description:

Objectives: This project is designed to study the effects of aging on the physiological responses to and recovery from exercise--to describe age changes and to elucidate the mechanisms of these effects of aging. It is designed to identify underlying factors in the limitation of work performance and reduced mechanical efficiency in older people. For this purpose, detailed evaluation of pulmonary function and pulmonary response to stressful agents are carried out. Other factors such as the metabolic cost of limb movement and psychomotor control of limb movement are being studied.

An additional goal is to identify and explain the role of disease-altered physiological function in age-related limitation of work performance. Cerebrovascular, cardiovascular and pulmonary disease and functional measures such as blood pressure, reflex time, and reaction time will be considered.

Methods Employed: Measured amounts of physical work are administered to subjects of varying ages by means of a calibrated arm ergometer and quantitative mechanical analysis of limb movement. A treadmill is used to induce higher levels of work. Measurements of oxygen uptake, CO_2 elimination, pulmonary ventilation volume, heart rate, blood pressure, and electrocardiogram are made before, during and after standardized amounts of exercise. The functional capacities of the pulmonary system are evaluated. Alterations in respiratory function as a result of acute, reversible airway constriction are evaluated by bronchial provocation techniques employing methacholine and ragweed antigen as challenge agents. Measurements of forced expiration (FEV), static lung volumes, plethysmographic conductance (SGaw) and inert gas (nitrogen) distribution are made using standing methods.

Major Findings: Pulmonary function was measured in seven subjects with ragweed allergic asthma, 7 subjects with ragweed hay fever and 7 non-allergic normals following bronchial challenge with methacholine. In comparing the responses of the three groups to methacholine we found that when we used measurements of SGaw, the asthmatic and hay fever groups were indistinguishable from each other and significantly more sensitive to the effects of methacholine than normals. On the other hand, when we used measurments of FEV_1, the normals and hay fever subjects were indistinguishable from each other and were significantly less sensitive than asthmatics. Residual volume measurements revealed significant increases for the asthmatics at very low dose levels with no change in the other two groups. Marked alterations in gas distribution and lung emptying were found in the asthmatics in the form of significant increases in the slope of the alveolar plateau while there were no observed changes in the other groups. In general, our findings supported the notion of a cholinergic hypersensitivity in allergic asthma that involved both peripheral and central airways. In addition the data demonstrates a similar hypersensitivity in the larger central airways in individuals with hay fever. The apparent discrepancy we observed in the group comparisons depending on whether we used measurements of SGaw or FEV_1 suggests that these tests reflect different sites of flow limitation. When the asthmatic subjects were challenged with an aqueous extract of ragweed antigen, we observed marked limitation of flow involving both central and peripheral airways, marked alteration of gas distribution within the lung and significant increases in lung volumes. These changes were not influenced by cholinergic blockade with atropine given prior to and during the challenge procedure, suggesting that cholinergic hypersensitivity and vagally mediated reflexes within the lung are not critical components of the airway response to inhaled antigen.

The Fitts type test of speed and accurancy of movement has been used to measure the information capacity of the eye-hand feedback loop in young

and old longitudinal studies participants. The test involves tapping by the subject alternately on two targets at maximum speed possible and consistent with a rather strict accuracy criterion. From trial to trial the targets are varied both in size and distance apart. Longitudinal changes were demonstrated for the first time for this kind of data. Analyses were performed for longitudinal studies participants 25 to 84 years of age. Subjects were classified for presence or absence of cerebrovascular disease by physical examination and careful review of pertinent medical history. Average rates of information gain were 8.0, 7,8, 7.8, 7.4, 6.7 and 6.2 bits per second for the age groups 25-34, 35-44, 45-54, 55-64, 65-74 and 75-84 years, respectively. On both a longitudinal and cross-sectional basis, significant changes occurred only after age 65. Longitudinal analysis showed that average rates of decline in information gain followed the cross-sectional trend at all age levels. In subjects over age 65 who had cerebrovascular disease, rates of decline in information gain were two to three times as great as for disease-free subjects. These age changes in motor control systems support our previous contention that loss of coordination is an important factor in reduced net mechanical efficiency of subjects over age 65.

Significance to Bio-Medical Research and the Program of the Institute: The pathophysiology of airway obstruction comprises a wide variety of anatomic and physiologic alterations. Because of the limitations of current methods of study, most of the physiological alterations are poorly if partially understood. Determination of the site of action in the lungs of allergenic agents, bronchodialators and bronchoconstrictors should permit appropriate evaluation and treatment of airway disease.

The decline of the ability of some older people to perform their day-to-day activities and to engage in pursuits which contribute to the economic and social strength of our society represents a national loss. Identification of the physiological, medical and social correlates of high levels of physical strength and psycho-motor performance in middle and old age, as well as declines in these abilities, should lead to techniques designed to reduce the rate of decline in performance capacities with age.

Proposed Course: Measurements of muscle strength and maximum power generating ability during arm exercise will be continued. Cardiovascular, ventilatory and metabolic responses to standardized arm ergometer exercise and monitored treadmill exercise will be used to classify participants into fitness categories and to explore the age relationships of biochemical and metabolic responses to exercise. Measurements of lung volumes and uniformity of pulmonary ventilation will be made to characterize the respiratory competence of the longitudinal studies participants. Measurement of respiratory drive in relation to various stimuli will be evaluated in these participants.

Keyword Descriptors: Age, Exercise, Lung Function, Motor Coordination

Honors and Awards: None

Publications: None

Serial No. Z01-AG-00017-17-CPB
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Age Relationships of Body Composition, Nutrition and Physical Activity

Previous Serial Number: HD-CP-25

Principal Investigators: Arthur H. Norris
Reubin Andres

Other Investigators: N. W. Shock, Paul J. Davis*, Marlene Butler, Gary Borkan**, Roger Aamodt***, Stanley M. Garn**

Cooperating Units: Department of Medicine*
Baltimore City Hospitals

Center for Human Growth & Development**
University of Michigan, Ann Arbor

Nuclear Medicine Dept., CC, NIH***

Man Years:

Total:	2.95
Professional:	.45
Other:	2.50

Project Description:

Objectives: This project is designed to describe age differences and age changes in body composition, nutrition, and physical activity. Mechanisms of interaction of these functions and behaviors will be sought. The relationship of these measurements to other physiological, psychological and biochemical variables will be examined.

Methods Employed: Height, weight, and body circumferences of longitudinal study participants are obtained by standard anthropometric methods. Roentgenographic and anthropometric estimates of skeletal mass are combined with height, weight, and body circumferences to provide an estimate of body fat. Other estimates of fat include skinfold thickness measurements and fat thickness measurements from X-rays. Indices of lean body mass include: (1) basal metabolic rate determinations, (2) twenty-

four hour urinary excretion of creatinine, (3) total body potassium, and (4) total body water and extracellular water determinations by indicator dilution. Nutrient intakes and activity calories are estimated from a diary and a self-administered questionnaire. All such measurements are repeated in the course of each subject's participation in the longitudinal program.

Major Findings: In any dietary survey, it is of concern to know how much the intakes of the nutrients will vary from one season to the next. In an effort to discern such variability in our dietary data, 159 longitudinal participants completed four seven-day diet diaries, each during a different season of the year. Seasonal comparisons were made for total calories, protein, fat, carbohydrate, alcohol, saturated fatty acids, unsaturated fatty acids, cholesterol, fiber, calcium, iron, vitamin A, thiamine, riboflavin, niacin, ascorbic acid and vitamin B-6. Only vitamin A, ascorbic acid and vitamin B-6 intakes varied to any significant degree. Daily mean vitamin A intakes were remarkably similar for the seasons, winter, spring and fall, but significantly lower than the mean intake for the summer. Intakes of ascorbic acid were lower than in the fall and winter than in spring or summer. The mean vitamin B-6 intakes were significantly lower in winter than during the other three seasons. Mean intakes exceed the Recommended Dietary Allowance for vitamin A and ascorbic acid, and are 75% of the recommendation for vitamin B-6. Although one might not expect any seasonal influence in food intake in a group of people of this high socioeconomic level, apparently the decreased availability of foods rich in these vitamins during the fall and winter months does influence the intake of these nutrients as they are calculated from the seven-day diary.

The activity questionnaire administered at each visit to participants in the longitudinal study has been analyzed for both longitudinal and cross-sectional results. Over 60% of men aged 25-44 and over 40% of men age 45-74 participate in some form of physical exercise. Even in the men over 75 years of age, 30% do some form of physical exercise or in other ways increase their activity levels. Since most of the participants worked at sedentary jobs, retirement for many has resulted in more time available for physically active pursuits such as gardening ,golfing, and bowling. This point is demonstrated by the results of a longitudnal analysis of the activity data from questionnaires. Cross-sectional comparisons of age decade groups identify the levels of caloric expenditure. There was a difference of 300 calories/day between the youngest participants (1200 cal/day) and the oldest, 75-84 years of age (880 cal/day). Intermediate age groups had intermediate activity levels. Longitudinal analysis showed declines in activity ranging from -7 cal/day/yr to -30 cal/day/yr for young and old subjects (25-54 years and 65-84 years). In marked contrast, subjects of retirement age (55-64 years) showed an increase of 9 cal/day/yr. Longitudinal analysis showed weight patterns to be associated with changes in activity in various ways. Young subjects (25-44 years old) increased weight (0.6 kg/yr) while reducing activity. Middle aged subjects had stable weights (slight increase of 0.07 kg/yr) in association with decreasing activity levels (-7 cal/day/yr for 45-54 year olds) and increasing activity levels(9 cal/day/yr for 55-64 year

olds). In the oldest subject (65-84 years) decline in activity was associated with decline in weight of -0.4 kg/yr. Failure of the oldest subjects to maintain body weight may become a deleterious factor for some individuals.

Measurements of the mid-shaft dimensions of the second metacarpal bone of the left hand of longitudinal participants have been made from X-ray films of the hand. There was no effect of Vitamin D supplementation or thiazide medication on CCT in these participants. Thus, only participants who had osteoporosis or a history of cortico-steroid or estrogen therapy were eliminated from the study. There is an increase in medullary diameter with increasing age. This results in a reduction in CCT (overall diameter minus medullary diameter). The cross-sectional difference in CCT ranged from 0.57 cm in 35 to 44 year old subjects to 0.47 cm in subjects 75 to 84 years old. Longitudnal changes for age decade groups agreed with the cross-sectional trend of about 7% per age decade. While this study agrees, in general, with previous studies of bone remodeling with age, this is the first demonstration of longitudinal changes with age in a large number (150) of subjectswith three or more measurements.

Significance to Bio-Medical Research and the Program of the Institute: Nutritional deficiencies in the aged are known to be common and are generally attributed more to the socio-economic deprivation of this group than to biological or physiological aging effects. The volunteers in the Longitudinal Study Group are not a deprived group--it may be characterized as upper-middle class and has a very high educational level. It, therefore, offers a unique opportunity to study nutritional status under very favorable conditions. The nutritional effects of biological aging per se may therefore be separated from what might be called "social aging."

Certain age changes in organ systems and various diseases are thought to be affected by diet, level of physical activity, and body composition. From the repeated assessment of these factors over time, it may be possible to determine their relative contributions to longevity and the maintanance of health and vigor in later life. Difficulties associated with obtaining retrospective estimates of eating habits, activity and body composition in the past make a prospective approach necessary for the collection of reliable information.

Proposed Course: Studies of diet, physical activity and body composition will continue. Data already collected will be further analyzed. Interactions of changes in body composition, food intake, food composition, kind and amount of physical activity, disease, and age will be examined. Specifically, body fat and lean body mass estimates, nutrient intakes and physical activity category will be used in an analysis of risk of cardiovascular disease and of rate of aging of several organ systems.

Keyword Descriptors: Age, Body Composition, Nutrition

Honors and Awards: None

Publications: None

Serial No. Z01-AG-00018-09-CPB
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Marital, Sexual and Social Factors in Aging

Previous Serial Number: HD-CP-22

Principal Investigator: C. Martin

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	1.00
Professional:	1.00
Other:	.00

Project Description:

Objectives: Major goals are to: (1) compare longitudinal subjects of diverse age for evidence of marital, sexual and social variables that vary in relation to age, (2) determine whether individual differences in sexual functioning may be related to social, physiologic or psychologic characteristics, and to (3) discover whether any aspect of prior marital, sexual or social experience may be associated with entities of disease or longevity.

Methods Employed: One one or another visit to GRC, each participant in the longitudinal study was asked to contribute an interview concerning his history of marriage and sexual activity. The investigator in making this request outlined study objectives, provided assurance of confidence and emphasized the voluntary nature of such a contribution. Questions were memorized along with whatever response categories were needed for the recording of responses. Chapters of the life history pertaining to residential, military, religious, educational, occupational and parental-home experience were reviewed before introducing questions relating to marital adjustment and sexual conduct. This initial questioning provided a variety of information not otherwise obtained, aided rapport and established certain reference ages against which earlier behavior could be oriented in time. At present, 634 subjects have completed interviews. The refusal rate has been 2.1 percent.

Major Findings: During the year, data analysis focused upon the second stated objective: that of exploring the significnace of experiential, physical and physiological characteristics of longitudinal subjects in relation to frequency of sexual activity. A premise of the inquiry was that important correlates of level of sexual functioning may reveal processes which contribute to to the decline in sexual vigor that accompanies aging.

Experiential Variables and Sexual Frequency: Numerous measures obtained by questionnaire and interview failed to achieve statistical significance in correlation with level of sexual activity for age groups 20-39, 40-49, 50-64 and 65-79. These data thus indicate that sexual frequency is independent of: age at marriage, religiosity of the subject, religiosity of parents, economic status of parental home, level of harmony within the parental home, times intoxicated before report, current hours of sleep, level of physical activity, as revealed by questionnaire and scores obtained on the Cornell Medical Index. However, low but significant correlations were found between frequency of sexual activity at ages 20-39 and age at first coitus ($r = -.19$), number of coital partners ($r = .27$), and the maximum number of coital events in anyweek of marriage ($r = .39$). None of these factors appeared significantly related to sexual frequency at older ages. On the other hand, the sum of all sexual events reported to occur between 20 and 40 years of age resulted in significant correlations with sexual frequencies at ages 40-49 ($r = .50$), ages 50-64 ($r = .40$) and ages 65-79 ($r = .20$). This latter finding indicates that respondents tended to maintain their relative levels of sexual activity over many years, in accord with the observations of other investigators.

Physical and Physiological Variables and Sexual Frequency: Again many physical and physiological attributes were found to be independent of level of sexual functioning; namely, height, weight, lean body weight, percent body fat, grip strength, abdominal circumference, prostatic size, triglycerides, hematocrit, hemoglobin, creatinine excretion, basal pulse rate, basal systolic and diastolic pressures, vital capacity and forced expiratory volume at one second (FEV_1). However, modest but significant correlations appeared between sexual frequency at ages 65-79 and chest circumference ($r = .27$), maximum breathing capacity ($r = .21$), BMR ($r = .22$), basal O_2zconsumption ($r = .26$) and serum cholesterol ($r = -.24$).

In sum, it was found that certain behavioral variables were associated with frequency of sexual activity at ages 20-39 but not at older ages, that respondents tended to maintain their relative level of sexual functioning over many years, and that the few physical and physiological attributes which appeared as correlates of sexual frequency in late age provide only limited support for the concept that physical fitness is important for the retention of sexual vigor in old age.

Significance to Bio-Medical Research and the Program of the Institute: While frequency of sexual expression in the male is known to be affected by age, marital status, potency and physical and emotional health, the

general questions of why males of comparable age vary widely in frequency of sexual activity and why the sexual vigor of the male invariably declines with age remain poorly understood. Although the initial premise that important correlates of sexual frequency might be found which are independent of age was not realized, the finding that many plausible areas of inquiry proved negative should help focus future investigation. Moreover, the demonstration that males tend to maintain relatively high or low levels of sexual activity over many years of their lives is consistent with the observation of Pfeiffer and Davis, and Masters and Johnson.

Proposed Course: Other factors of possible importance for level of sexual functioning remain to be explored. In this connection, the smoking history, dietary habits, neurophysiological function and plasma testesterone levels (in collaboration with Dr. S. Mitchell Harman) will be considered. In addition, analyses are projected with reference to the third stated objective. In this endeavor, subjects with diagnoses of cornomary artery disease, hypercholesterolmia and other conditions of unknown etiology will be compared with subjects of similar age without these conditions, for evidence of differences with respect to marital, sexual and social background factors.

Key Word Descriptors: Aging, Sexual Behavior, Sexual Interview

Honors and Awards:

Dr. Martin was invited to co-chair a symposium on "The Sex LIfe of the Aging Person" planned for the 10th International Congress of Gerontology in June, 1975. A paper summarizing the above described study was prepared for presentation.

Dr. Martin discussed "Sexuality in the Older Male" before the Family Planning Training Institute of the Planned Parenthood Association of Maryland on September 23, 1974.

Publications: None

Serial No. Z01-AG-00019-12-CPB
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Dermatoglyphics and Haptoglobins in Clinical Medicine and Aging

Previous Serial Number: HD-CP-13

Principal Investigator: Chris C. Plato

Other Investigators: W. Wertelecki*
J. D. Niswander**
F. S. Steinberg***
J. Cereghino†
H. Fessas††

Cooperating Units:
Department of Pediatrics* University of Southern Alabama
Department of Human Genetics, NIDR**
Program Statistics Branch, NICHD***
C & F Research Center, NINDS†
Nicosia, Cyprus††

Man Years:

Total:	.80
Professional:	.50
Other:	.30

Project Description:

Objectives: 1. To investigate possible differences in the frequency distribution of the haptoglobins and dermatoglyphic patterns between children and adults. 2. To establish possible associations between various clinical anomalies and dermatoglyphic features. 3. To establish suitable normal control panels, Caucasian and Negro and male and female, which may be used in future studies to determine associations between dermatoglyphics and diseases or to be used as normal controls for comparison with older individuals, in aging studies including those of the GRC longitudinal study.

Methods Employed: Sera and prints were collected from each participant of the longitudinal study. The sera were evaluated for the main haptoglobin types through starch gel electrophoresis. The Hp 1-1 or Hp 2-1 will further be subtyped through column electrophoresis. Dermatoglyphics

were also collected from patients with leukemia, Down's syndrome, congenital heart and other diseases, and their sibs and parents. Normal controls were obtained from seven year old Caucasian and Negro, male and female, participants of the Perinatal Study Branch, at Boston and Baltimore.

Major Findings: We established the only available normal control panels (for Caucasian and Negro, male and female) on dermatoglyphics. These may be used by us and other researchers (a) to test dermatoglyphic associations with diseases and (b) to be used as controls for studying the dermatoglyphic frequencies in older individuals (since they are all 7 years old). A pilot study (presented in Bratislava) indicated significant differences in the Simian and Sydney creases between the seven year olds and adult married individuals. The biological significance of these observations is presently being evaluated. Associations were also found between dermatoglyphics and leukemia, Down's and 18q- Syndrome. The serum protein as well as the dermatoglyphic data from the Gerontology Research Center participants are presently in the collection and evaluation stage.

Significance to Bio-Medical Research and the Program of the Institute: The finding of significant statistical associations between various diseases and dermatoglyphic markers (1) may assist in the determination of genetic factors in these diseases. (2) Provide appropriate normal controls for both clinical and age-related dermatoglyphic studies. (3) Compare possible quantitative or qualitative haptoglobin changes with age.

Proposed Course: To carry through the haptoglobin and dermatoglyphic evaluations on the GRC Longitudinal Study subjects. To continue this method of investigation taking into consideration other clinical anomalies and family units and their controls.

Key Word Descriptors: Aging, Dermatoglyphics, Haptoglobin, Medical Genetics

Honors and Awards:

Mr. Plato organized and chaired the dermatoglyphics section of the Annual Meetings of the American Society of Human Genetics, in Portland, Oregon.

Mr. Plato presented two invited papers at the I International Congress of Twin Studies in Rome, Italy.

Mr. Plato presented an invited paper at the 6th Bartos Symposium on Dermatoglyphics in Bratislava, Czechoslovakia.

Mr. Plato was elected Chairman of the Committee for organizing the American Dermatoglyphics Association.

Publications:

Plato, C. C., Cereghino, J. and Steinberg, F. S.: The dermatoglyphics of American caucasians. Special issue of the American J. Physical Anthropology, 42:195-210, 1974.

Plato, C. C. and Wertelecki, W.: The dermatoglyphics of American caucasian adults. In: The Proceedings of the 6th Bartos Symposium on Dermatoglyphics. Bratislava (Smolenice), Czechoslavaki, October 21-23, 1974. In press.

Serial No. Z01-AG-00020-12-CPB
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Twin Studies

Previous Serial Number: HD-CP-14

Principal Investigator: Chris C. Plato

Other Investigators: J. T. Schwartz*
W. Wertelecki**

Cooperating Units: F. & D. Research, NEI*

Department of Pediatrics**
University of Southern Alabama
Mobile, Alabama

Man Years:

Total:	.20
Professional:	.20
Other:	.00

Project Description:

Objectives: 1. To determine the degree of variability and heritability of the structure of the human iris. 2. To investigate through cross-sectional twin studies the possible structural and pigment differences in the iris of individuals in different age groups. 3. To develop, through dermatoglyphics and iris structure, further criteria for twin zygocity diagnosis. 4. To determine the genetic vs. intrauterine effect on the formation of dermatoglyphics and their association with disease.

Methods Employed: Thorough eye examinations, color slides of the irises and finger and palm prints, data were obtained from over 600 pairs of twins varying from new born to the age of seventy years old. Zygocity was estabilised by elaborate blood group typing, perinatal information, and physical similarities. In addition to the twins we are also including in this study 250 pairs of sibs to serve as controls.

Major Findings: Through our twin and sib studies we demonstrated that contrary to common belief dermatoglyphics, even though genetically determined, are to a great extent influenced by intrauterine factors during the early stages of preganancy. Various dermatoglyphic traits

are variably affected by these intrauterine factors. The iris studies are still under investigation and the data are still being evaluated. The results of these experiments become of importance when one considers the etiology of diseases such as Down's Syndrome, 18 q Syndrome, leukemia and others which have been shown to be associated with dermatoglyphic peculiarities.

Significance to Bio-Medical Research and the Program of the Institute: 1. To establish possible associations between the iris structure and eye disorders. 2. To establish possible effects of age upon the structure and pigment of the human iris and possible association with age-related eye diseases. 3. To offer additional means for determining twin zygocity. 4. To establish hereditary patterns in the transmission of iris structure and dermatoglyphic features. 5. To determine the effect of intrauterine environment in the determination of dermatoglyphics.

Proposed Course: To evaluate the data already collected and to perform the appropriate statistical tests.

Keyword Descriptors: Twins, Genetics, Iris Structure, Dermatoglyphics

Honors and Awards: See Project No. Z01-AG-00019-12-CPB

Publications: None

Serial No. Z01-AG-00021-12-CPB
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Dermatoglyphics of Primitive and Other Populations

Previous Serial Number: HD-CP-15

Principal Investigator: Chris C. Plato

Other Investigators: D. Carleton Gajdusek*
Ralph Garruto**
Robert MacLennan†
H. Brown***
Marshall T. Newman††
R. W. Hornabrook†††

Cooperating Units:

C & F Research, NINDS*

Special Chronic Disease Studies**
Department of Anthropology
Pennsylvania State University

International Agency for Research on Cancer†
WHO, Lyon, France

Department of Anthropology††
University of Washington
Seattle, Washington

Port Moresby, New Guinea***

Institute of Medical Research†††
Goroka, Papua, New Guinea

Man Years:

Total:	.50
Professional:	.30
Other:	.20

Project Description:

Objectives: This project represents part of a multidisciplinary effort in conjunction with the World Health Organization and other institutions to study various genetic, clinical and anthropological markers among the developing peoples of the South Pacific and South America. Our part is to establish the distribution patterns of the dermatoglyphic features among the various populations and geographical areas, and map them in such a way as to serve as reference and source of comparison.

Methods Employed: Dermatoglyphics were collected by the Faurot inkless method. Data were evaluated. Our main area of study is South Pacific and Australasia in general, Peru, Colombia, and Mexico.

Major Findings: Dermatoglyphic data which have been analyzed have been reported in the literature. Four papers listed as in press last year have now been published. Additional dermatoglyphic data have been collected. Analysis is in progress.

Significance to Bio-Medical Research and the Program of the Institute: The dermatoglyphic frequency distribution atlas and other data are being used by a number of investigators for genetic or clinical dermatoglyphic studies. Furthermore, the thorough evaluation of genetic markers (including dermatoglyphics), of the few remaining primitive populations will allow scientists to ascertain the effect of "Western" culture, infectious and environmental pollution upon the overall health and especially some of the diseases of middle age and beyond, which we often consider as the results of Westernization, i.e. atherosclerosis, high blood pressure, etc.

Proposed Course: Data for isolated populations of New Guinea, New Hebrides, Solomon Islands, Yap and Marshall Islands are being evaluated for comparison with populations previously reported and to identify other genetic markers.

Keyword Descriptors: Primitive Populations, Dermatoglyphics, Genetics

Honors and Awards: See Project No. Z01-AG-00019-12-CPB.

Publications: None

NICHD Annual Report
July 1, 1974 through June 30, 1975
Gerontology Research Center
Laboratory of Cellular and Comparative Physiology

Research activities were centered about the problem on age effects on modulation of proliferation and differentiation at the cellular and molecular levels. Aging of the immune system and aging of the fibroblasts constituted about 70 and 25 percent of our effort, respectively, and another 5 percent of our effort was devoted to two minor projects.

Current studies on aging of the immune system can be categorized into three areas: (a) cellular and molecular etiology of immunosenescence, (b) immunopathogenesis of aging, and (c) immunoengineering.

A. Cellular and molecular etiology of immunosenescence

Previous studies have revealed that aging may be selectively affecting the T cell arm of the immune system. Therefore, much of our current effort has been expanded towards the effects of age on the differentiation of T cells.

Studies on stem cells were centered about age effects on their proliferative capacity. The results of this study in progress indicate that the proliferative capacity of old stem cells grown in old mice is only about 10% that of young stem cells grown in young mice, due in part to age-related changes in the cellular environment and in part to age-related changes within the stem cells.

Because the thymus is responsible for the differentiation of stem cells into T lymphocytes, and because its involution heralds the decline of certain T cell-dependent immune functions, studies were initiated to assess the differentiation capacities of thymic tissues of aging mice. These results indicate that certain subpopulations of T cells are generated only during growth of the individual, whereas others are generated throughout life.

Studies on the regulation of immune response showed that a plastic-adhering "sticky" cell exists in the spleen that is capable of regulating T cell-dependent but not T cell-independent humoral immune response. The cell has been classified as a B cell based on its morphology, its responsiveness to mitogens, and by the presence of membrane surface immunoglobulin receptors.

Studies on the effects of age on membrane structures revealed that (a) lymphocytes of old mice appear to be more fragile than lymphocytes of young mice as judged by their susceptibility to antiserum reagents, (b) enriched T cell fractions from young mice respond to T cell-specific mitogen, PHA, by generating a cyclic-GMP level four times higher than that of old mice, and (c) cyclic-AMP can stabilize lymphocyte membranes.

One of the objectives of our membrane structure studies is to correlate morphologically distinct cellular characteristics with distinct functions for each subpopulation of cells of the immune system. To this end effort has been devoted to improve the existing techniques. The results have been gratifying. A new tri-labelling technique was developed for scanning electron microscopy which permits unambiguous identification of subpopulations of cells and quantitation of distinct cell surface receptors on individual cells. Moreover, through use of soluble membrane antigens, it was demonstrated that the commonly employed mixed lymphocyte culture (MLC) assay is in actuality an interaction between cells and is not dependent on antigenic recognition per se. It also explains why cells from immunodeficient individuals which respond poorly in most assays will respond vigorously in a MLC. The endotoxin, LPS, which is being used as a B cell probe, was shown to stimulate T cells under conditions where T cells are forced to enter cell cycle, indicating that LPS should be used more selectively.

Studies on the recently initiated molecular etiology of aging centered about the effects of aging on the fidelity of B cells. Antigen-induced specific antibodies of IgG_1 and IgG_2 isotypes obtained from 3 month and 2-3 year old inbred guinea pigs are being analyzed by various physicochemical parameters. The results indicate that the differences appear to be minimal, confirming the earlier observations based on cellular analysis of aging mice. To complement the B cell study, experiments were also carried out to assess the nature of decline in liver aldolase activity in mice. Evidence from studies in progress shows that the specific activity of the purified enzyme declines with age. Immunological analysis of the purified enzyme preparation shows that the old enzyme preparation contains two antigens, one of which is immunologically identical to the young liver aldolase. It remains to be established if the second antigen contains enzyme activity.

Studies on terminally differentiated T cells have been concerned with T killer cells in individuals with Hodgkin's disease. Kinetic phase light microscopic and scanning electron microscopic analyses revealed that they cytolyze autologous neoplastic Reed-Sternberg cells by affixing the tips of their microvilli on to target cells and subjecting the target cell membranes to shearing and tearing forces.

A study was initiated recently to complement our ongoing studies on terminally differentiated cells. It deals with an important, yet little understood, problem in aging, and that is how scavenger macrophages of the immune system recognize and remove their own terminally differentiated aging cells from the body. Evidence from studies in progress has proven both by direct and indirect methods that macrophages can differentiate adult self from senescent self on the basis of selective IgG isotype attachment to the surface of senescent cells. Young red blood cells (RBC) aged artificially in vitro by pretreating them with neuraminidase were phagocytized as rapidly as in situ aged RBC. This suggests that carbohydrate moieties are being exposed as RBC age naturally and, once exposed, these receptors are bound by pre-existing autoantibody-like Ig. The results indicate that the molecular basis of recognition of senescent cells by macrophages is immunological.

Future stem cell studies will determine (a) whether the decline with age in the proliferative capacity of old stem cells is reversible or permanent. Studies on the differentiation capacity of thymic epithelial tissue will center about its effects on lymph node and spleen seeking cell-mediated and regulatory T cells. Concerning the regulatory B cells, attempts will be made to determine their role, if any, in cell-mediated immunity. Membrane structure studies will include further morphological and functional characterizations of membrane changes associated with aging of various subpopulations of T cells. Expansion of our technical ability to measure functions may be in order, with the hope that better diagnostic criteria can be developed to evaluate immune functions. In this regard our human cross-sectional and longitudinal studies will focus on which of the multitude of indices may have predictive values. Molecular aging studies will continue to focus on characterization of macromolecules whose activities are altered with age. Finally, terminally differentiated cells will be studied with the scanning electron microscope to further characterize the membrane receptors which bind immunoglobulin G.

B. Immunopathogenesis of Aging

Aging mice and rats were used for this program. Studies with the mouse were focused on cellular characterization of naturally occurring and artificially induced immunodeficiency diseases. Characterization of the immune system of aging NZB mice which have a propensity to succumb to immunodeficiency diseases, revealed that (a) the development of cytotoxic T lymphocytes decreases with age, (b) the number of suppressor cells which can be generated with a mitogen decreases with age, and (c) cytotoxic T lymphocyte are distinct from mitogen generated suppressor cells. These results suggest that a breakdown in the regulatory mechanism may be involved in the initiation of autoimmune disease of the short-lived NZB mice. Characterization of artificially induced, immunologically crippled, allogeneic bone marrow chimeras revealed that those reared in a laminar flow environment are distinct from those reared in a germfree environment. The main difference is the histocompatibility (H-2) type of lymphocytes. Only donor type was seen in germfree reared chimeras, but donor type, host type, and donor-host types were seen in laminar flow reared chimeras. These results indicate that the allogeneic bone marrow chimeric state is not due to tolerance as suspected by many in the past.

Previous studies of aging Wistar rats revealed that the kidney disease prevalent among these rats has a close relationship with age-related decline in immunologic activity. Therefore, a more systematic study was initiated to determine whether the rat can be used as a model animal with which to study pathogenesis of aging. Our initial effort was expanded primarily on the development and improvement of <u>in vitro</u> assays of rat cells. These studies show that rat cells are more difficult to manipulate than mouse and human cells. However we are hopeful that eventually the rat will be amenable to cross-sectional and longitudinal analyses.

C. Immunoengineering

Three broad approaches have been undertaken since the inception of this program a year ago: chemical therapy, cellular therapy, and dietary manipulation.

In our chemical therapy program we have assessed the effects of two chemicals: 2-mercaptoethanol, a reducing agent, and thymosin, a compound presumably synthesized by the thymic epithelial tissue, and, therefore, the hormone responsible for transforming stem cells into T cells.

The results with mercaptoethanol showed that both the proliferative capacity and humoral helper function of old T cells can be markedly enhanced with 2-mercaptoethanol. In contrast, bovine thymosin, which was kindly supplied by Dr. Allen Goldstein, Division of Biochemistry, University of Texas, Galveston, Texas, had no appreciable enhancing effect on both the T and B cells following its injection into old mice, an injection schedule of Goldstein which was found to be effective for short-lived, autoimmune-prone NZB strain of mice.

Our recently initiated cellular therapy program has been centered about the effects of young stem cell infusion and newborn thymus graft on immune functions of old mice. It would appear that old mice given the combination therapy of young stem cell injection and newborn thymus graft have an elevated T cell-dependent humoral immune activity for at least 4 months after the treatment.

Because it has been well established that dietary manipulation allows certain mammals to live longer than their normal apparent life span, a study was initiated to measure the effects of five protein restricted dietary regimens on the life span and immune activity of mice. Immunological and biochemical studies will be initiated early next fiscal year. The recently determined measurements of body weight and survival indicate that aging mice subjected to a low protein diet (4%) at weaning are small and have a shorter life expectancy.

Studies on human fibroblasts were carried out to: (a) determine what cellular processes become limiting as they age in tissue cultures and in situ, (b) relate genetic alteration, aging and malignancy, and (c) elucidate the effects of aging on regulatory gene functions.

Serially passaged culture studies revealed that as fibroblasts age in vitro (a) they become bigger, heavier and less proficient in their ability to proliferate, (b) the variation in DNA content of individual cells increased and (c) large cells were distributed in all phases of the cell cycle. These characteristics were induced in mitotically active, small, young fibroblasts by culturing them in serum-deficient medium and by exposing them to hydroxyurea, an inhibitor of cellular proliferation. It would seem that an increase in cell size and density may be a manifestation of cells becoming less proficient in their proliferative ability.

Cells from patients with Gardner's syndrome, a premalignant genetic disorder, revealed a marked increase in the frequency of chromosomal aneuploidy when compared with cells from age-matched controls (i.e., 24.4% versus 9.2%).

Studies on chromosome 21 directed antiviral genes revealed that (a) the regulatory gene controlling the antiviral gene is located on a chromosome

other than chromosome 21, (b) no change in the regulation of antiviral gene was observed in fibroblasts aged in vitro, although changes were observed in fibroblasts obtained from aged donors, and (c) a repressor gene can mediate its action through complex interchromosomal gene interactions.

Additional activities were centered about two other areas: maternal age effects and regulation of genetic messages during early development.

Studies on the development of the mouse as an animal model for the effects of maternal age on chromosomal disorders indicate that with increased maternal age the frequency of chromosomally abnormal fetuses increases from 3% to 15%. Mosaicism and trisomies are the most frequently observed forms of chromosome abnormality. A good correlation has also been observed between morphologically abnormal fetuses and chromosomal abnormalities.

Studies on messenger regulation during development of sea urchin eggs have been extended to include parameters such as enzymatic factors involved in the post-fertilization recruitment of pre-existing genetic information. Highlight of recent studies is the demonstration that suppression of post-fertilization protein synthesis did not suppress polyadenylation. This suggests that post-fertilization protein synthesis could be a prerequisite to the selection of specific genetic message.

The Laboratory of Cellular and Comparative Physiology has completed its third year under a new set of goals directed toward (a) an understanding of the effects of age on the cellular and molecular mechanisms responsible for the deterioration of certain cells of the immune and related systems, (b) the development of methods for early detection of signs of cellular aging and (c) the development of methods to control harmful changes. Associated with the change in laboratory goals, there has been a gradual change in the organizational structure of the laboratory geared towards an effective interaction between working groups. The acquisition of a scanning electron microscope should enhance such an interaction, especially between the cellularly and molecularly oriented groups.

Serial No.: Z01-AG-00081-03-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project title: Age Effects on Proliferation and Differentiation of Immune Cells

Previous Serial Number: HD-CCP-24

Principal Investigator: T. Makinodan

Other Investigators: K. Hirokawa
M. M. B. Kay
G. H. Stoltzner
P. A. Barstad

Cooperating Units: University of Texas at Galveston
Galveston, Texas

University of Nebraska
Omaha, Nebraska

Tokyo Medical and Dental University
Tokyo, Japan

Man Years:

Total	2.85
Professional:	1.25
Others:	1.6

Project Description:

Objectives: The proliferative and differentiation capacities of immunocompetent cells decrease with increasing age. Consequently certain normal immunologic functions decline. Functional alteration of precursor cells may reflect a genetically programmed postmaturation event or a stochastic event. It may also reflect a change in the proportion of regulator cells with suppressive and enhancing activities. The objectives of this project are (a) to determine the mechanisms responsible for functional alterations of immunocompetent precursor cells and (b) to develop methods for controlling age-related decline in normal immunologic functions.

Methods Employed: The proliferative capacities of bone marrow stem cells of young and old mice were assessed in vivo by determining their colony forming and proliferative activities in young and old recipients.

Stimulation of enriched T cells of young and old mice in response to specific mitogens was assessed for changes in the level of cyclic-GMP and cyclic-AMP. The T cell-transforming capacity of thymic epithelial tissues was assessed by implanting into T cell-deficient young mice thymic grafts of donor mice ranging in age from one day to 33 months and then by determining splenic T cell activities at varying intervals. The effects of bovine thymosin fraction 5 and a control, brain extract fraction 5, were assessed by measuring the blastogenic response of spleen, lymph node and bone marrow cells of young and old mice following 5 consecutive injections of the hormone at a dose of 4 mg per injection. The effects of mercapto-ethanol on young and old mice were assessed in terms of the ability of their spleen cells to mount a T cell-dependent humoral immune response and a blastogenic response. Attempts to enhance T cell immune functions of old mice were made by grafting newborn thymic tissues, by injecting young adult stem cells, and by the combined newborn thymic graft-young stem cell injection treatment.

Major Findings:

1. The proliferative capacity of old stem cells grown in old mice is 10% that of young stem cells grown in young mice, and this is due to age-related changes in the stem cells and in their environment.

2. Enriched splenic T cells of young mice respond to PHA by generating 2-4 times higher levels of cyclic-GMP than those of old mice, but the levels of cyclic-AMP were about the same.

3. Certain T cell differentiation capacities decreased abruptly and early in life, whereas others seem to continue throughout life but with a declining efficiency.

4. Bovine thymosin had no appreciable enhancing effect on the proliferative capacity of T and B cells of old mice.

5. Both the proliferative and humoral helper activities of old T cells can be enhanced significantly with mercaptoethanol treatment.

6. The combination young stem cells injection-newborn thymus graft therapy can elevate significantly the T cell immune function of old mice for at least 4 months.

Significance to Biomedical Research and the Program of the Institute: The decline of functional immune activities with age has an obvious effect upon senescence in general and extension of health. If the cell types and regulator factors which are responsible for the decline can be identified and characterized, this will be a significant step toward determining the cause for the decline and approaches to control the decline. Moreover, a comprehensive understanding of the effects of aging on the immune system will contribute to other vital tissue and organ systems, especially those involving cells undergoing proliferation and differentiation.

Proposed Course: Efforts will continue to focus on the mechanisms responsible for functional alterations of immunocompetent precursor cells and on the development of methods for contolling the decline in normal immune functions.

Honors and Awards:

Dr. Makinodan was invited to give a lecture at the annual "Clinical Day" of the Jewish General Hospital, Montreal, Canada, on October 10, 1974.

Dr. Makinodan was invited by the Science & Technology Agency, Japanese Atomic Energy Commission, Tokyo, Japan, to give a series of lectures in Tokyo, Kyoto, and Hiroshima on the biology of aging, immunity and aging, current topics in cellular immunology and late radiation effects on the immune system, March 3-19, 1975.

Dr. Makinodan was selected to receive the annual Biomedical Sciences Award for 1975-76 by the Andrus Gerontology Center, University of Southern California, Los Angeles, California.

Publications:

Goodman, S. and Makinodan, T.: Effect of age on cell-mediated immunity in long-lived mice. Clin. Exp. Immunol. 19: 533-542, 1975.

Gottlieb, C. and Makinodan, T.: T-cell radiation sensitivity during immune interaction. Discussion remarks. In Interaction of Radiation and Host Immune Defense Mechanisms in Malignancy. Upton, N. Y., Brookhaven National Lab., BNL 50418, 1974, pp. 258-263.

Hallsall, M. K. and Makinodan, T.: Analysis of the limiting-dilution assay used for estimating frequencies of immunocompetent units. Cell. Immunol. 11: 456-465, 1974.

Hirokawa, K. and Makinodan, T.: Thymic influence on T cell differentiation. J. Immunol. In press.

Makinodan, T.: Cellular basis of immunosenescence. INSERM 27: 153-165, 1974.

Makinodan, T. and Adler, W. H.: Effects of aging on the differentiation and proliferation potentials of cells of the immune system. Fed. Proc. 34: 153-158, 1975.

Makinodan, T., Heidrick, M. L. and Nordin, A. A.: Immunodeficiency and autoimmunity in aging. In Bergsma, D., Good, R. and Finstad, J. (Eds.): Immunodeficiency in Man and Animal. Stamford, Conn., Sinauer Press, 1975, pp. 193-198.

Stoltzner, G. and Makinodan, T.: Age dependent decline in proliferation of lymphocytes. In Proceedings of the Philadelphia Symposium on Aging, Valley Forge, Pa., September 30-October 2, 1974. In press

Vasquez, J. and Makinodan, T.: Aging and the immune system. A brief summary of current knowledge. In Ostfeld, A. M. and Gibson, D. C. (Eds.): Epidemiology of Aging. Washington, D. C., U. S. Government Printing Office, 1975, DHEW Publ. No. (NIH) 75-711, pp. 161-173.

Serial No.: Z01-AG-00082-01-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Analyses for Age-Related Changes in Humoral Antibodies of Guinea Pigs

Previous Serial Number: None

Principal Investigator: Peter H. Koo

Other Investigators: None

Cooperating Units: Department of Biology
Johns Hopkins University
Baltimore, Maryland

Man Years:

Total:	1.05
Professional:	1.0
Others:	0.05

Project Description:

Objectives: To determine if there are age-related changes in the quality of humoral antibodies of IgG and IgM isotypes with respect to (a) their affinities for a series of structurally related and unrelated haptens and antigens, (b) their topographic structures of antibody combining sites or idiotypes (variable region sequences), (c) their possible changes in constant region sequences due to somatic mutations, (d) the degree of cross-reactivity with structurally similar haptens, and (e) the degree of cross-reactivity between a specific hapten with two or three different antibodies of different specificities, produced by animals immunized with two or three structurally related antigens.

Methods Employed: Naturally raised antibodies, such as anti-p-azobenzene-arsonate antibodies of IgG2 and IgG1 isotypes, obtained from young and aging (2-3 year old) inbred strain 13 guinea pigs are being studied. Equilibrium dialysis techniques were used for determining binding constants and heterogeneity indices of specific antibodies for haptens. Affinity labeling techniques were used for probing the combining site structures of antibodies. The structural differences between old and young antibody molecules are to be determined by peptide-mapping, by primary structural sequence determination either by automatic protein sequencer or by manual Edman degradation

procedures, by amino acid compositional analyses, by isoelectric-focusing on polyacrylamide gel.

Major Findings:

1. The binding constants of IgG2 and IgG1 anti-p-azobenzenearsonate antibodies for N-acetyl mono(p-azobenzenearsonate)-L-tyrosine appear to be only slightly higher for young than those of aging guinea pigs.

2. In both young and old IgG1 antibodies, same type of amino acid residues (lysine and tyrosine) are modified by an affinity labeling reagent N-bromoacetyl mono(p-azobenzenearsonate)-L-tyrosine, though IgG1 of young guinea pigs seems to have a slightly higher number of lysine residues modified than IgG1 of aging guinea pigs, suggesting that the IgG1 antibodies of both young and aging guinea pigs have similar topographical regions in the combining sites except for some possible minor structural changes.

3. A CNBr peptide fragment from the heavy chain of IgG1 antibodies, which contains the "second hypervariable region" of the H-chains, has been isolated and partially sequenced. The results obtained from the automatic sequencer are still being analyzed but, thus far, no major sequence difference has been confirmed.

4. Amino acid compositions of comparable peptides isolated from antibodies of 2 to 3 year old NIH guinea pigs and 3-4 month old JHU guinea pigs are different only in certain particular residues, indicating some possible changes in the primary structures of antibodies with age.

5. IgG1 and IgG2 antibodies of young and aging guinea pigs do not seem to be less heterogeneous than those of the young as judged by isoelectric-focusing gel.

6. The purified IgG2 fractions of the older animals which have been deprived of specific antibodies contain homogeneous proteins not found in the young animals. The possibility that these homogeneous proteins being inactive antibodies or idiopathic paraproteins of IgG2 subclass is being investigated.

Significance to Biomedical Research and the Program of the Institute: One of the general phenomena observed in the aging animals is that the antibody titers decrease with the age of the animal. This decrease in the antibody titer could be due to several sources, among which are (a) a decline in high affinity antibodies with an increase in low affinity antibody molecules which are not being accurately measured by conventional measurements; (b) loss of antibody function (i.e., emergence of inactive antibody); (c) changes in antibody specificities or idiotypes of the antibodies. Elucidation of the cause(s) of decline in antibody titer will provide us with one approach to the effects of age on the immune system.

Proposed Course: Physico-chemical analyses of IgG1 and IgG2 antibodies of young and aging inbred guinea pigs will continue along the lines described here.

Keyword Descriptors: Inbred strain 13 guinea pigs, antibody avidity, specificity and cross-reactivity, affinity labeling, equilibrium dialysis, isoelectric-focusing gel analysis, inactive antibody, idiopathic paraproteins, automatic sequencer, antibody heterogeneity, peptide mapping, antibody combining sites, amino acid compositions.

Honors and Awards: None

Publications: None

Serial No.: Z01-AG-00083-01-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Age Related Changes in Mouse Liver Aldolase

Previous Serial Number: None

Principal Investigator: Celine Tan

Other Investigators: T. Makinodan

Cooperating Units: None

Man Years:

Total:	1.6
Professional:	1.0
Others:	0.6

Project Description:

Objectives: Several enzyme systems exhibit the phenomena of age-related changes in their activity. This change in enzymic activity with age could be of great biological significance for it provides a sensitive indication of the basic changes with age in metabolism of tissues. However, the nature of the changes have not been elucidated. Our aim is to ascertain the nature of the change in liver aldolase, an enzyme involved in glucose metabolism, in aging long-lived BC3F1 mice.

Methods Employed: Liver aldolase of 1 to 1-1/2 month, 2-4 month and 22-36 month old mice was isolated and purified according to the method of Gracy et al. (Arch.Biochem.Biophys. 136:480, 1970). Purification consisted of chromatography on phosphocellulose columns, ammonium sulfate precipitation and a rechromatography on phosphocellulose with substrate elution.

The purified enzymes were characterized in terms of their mobility on polyacrylamide gels, enzyme kinetics, heat stabilities, and fingerprinting of tryptic peptides on thin layer cellulose plates. Antiserum reagents were prepared against the purified aldolase of young and old mice, in rabbits and tested by immunoelectrophoretic and immunodiffusion analyses.

Major Findings:

1. Enzyme purified from young mice liver showed consistently a higher specific activity than that enzyme purified from old mice liver (2 to 3 fold).

2. Kinetic measurements showed Km to be unchanged but Vmax was much lower for enzymes from old liver.

3. Enzyme purified from old mice liver was more heat labile.

4. Tryptic peptide maps revealed peptide differences between enzyme from young and old liver.

5. Immunochemical analyses revealed that the purified aldolase preparation from old mouse liver contained two major antigens.

Significance to Biomedical Research and the Program of the Institute: Age-related decline in mouse liver aldolase activity may be a suitable model to analyze molecularly, whether the change reflects primarily an alteration at the transcriptional, translational or post-translational level.

Proposed Course: Immunochemically pure preparation of liver aldolase of young and old mice will be analyzed for differences in their primary structure. Aldolase of other tissues and other strains of mice will be analyzed in a similar manner.

Honors and Awards: None

Publications: None

Serial No.: Z01-AG-00084-01-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Molecular Genetics of Aging and Regulation

Previous Serial Number: None

Principal Investigator: Y. H. Tan

Other Investigators: Nancy Lundh

Cooperating Units: None

Man Years:

Total:	2.5
Professional:	1.7
Others:	0.8

Project Description:

Objectives: To establish an experimental model for the elucidation of the regulatory gene functions in human cells and their studies as a consequence of age. Specifically, we plan to investigate: (a) the regulation of chromosome 21-directed anti-viral gene(s) in man; (b) the fidelity of this regulation as a function of age; (c) the assignment of this regulatory gene function to a specific chromosome; and (d) the hypothesis that the increased incidence of oncogenic diseases (Tan, et al., Science 186:61, 1974) is related to changes in the regulation of the anti-viral gene(s).

Methods Employed:
1. We shall utilize the parasexuality of somatic cell hybrids to investigate the regulation of chromosome 21-directed gene products. For further description of this approach, refer to Tan, Nature 253:280, 1975; Tan, et al., PNAS 71:2251, 1974; Tan, Linkage analysis of animal cell virus interaction in man by somatic cell genetics, Symposium on Interferon Regulation and Action, Academic Press, 1975, in press.

2. We will test the preservation of this function as a consequence of (a) in vitro aging viz. senescence in tissue culture and (b) cells derived from old human donors.

3. We will use the procedure in (1) to perform the assignment.

4. To investigate this hypothesis, we will compare the replication of viruses in old and young cells. The old cells which have been postulated to be constitutive for the anti-viral genes should support less virus replication. Vesicular stomatitis virus and herpes simplex will be used as an index to monitor these parameters.

Major Findings:

1. We have obtained evidence that the regulator gene which controls the structural gene which codes for the anti-viral protein is located on a separate chromosome.

2. We have obtained evidence suggesting the existence of a repressor gene which mediates its action through interchromosomal gene interaction.

3. We have identified no changes in the regulation of the anti-viral genes as a consequence of in vitro aging (Hayflick's phenomena) although changes were observed with the inducibility of the anti-viral gene(s) from fibroblasts obtained from donors of different ages.

4. We have identified 4 cell lines which will allow us to design experiments to test the hypothesis that transformability of human cells is influenced by the constitutiveness of the anti-viral genes.

Significance to Biomedical Research and the Program of the Institute: These lines of research should lead to the development of a number of molecular models for investigating the genetic and epigenetic conditions that are associated with the aging phenomenon. The proposal on genetic regulation should be of interest to the biomedical researcher because numerous disease states can now be investigated from the level of breakdown of cellular regulation caused by disruption of those mechanisms responsible for the maintenance of cellular homeostasis. The proposal on the molecular genetics of viral replication and transformation in old human cells is of vital importance to our understanding of the higher incidence of cancer in the aged. This may be explained on the basis that the oncogenic potential of a virus is increased when its proliferative replication is decreased. Regardless, our proposal should provide opportunities for applying basic hypothesis to explain the various virally associated pathologic conditions in the aged.

Proposed Course: During our first year, we have set up the rudiments of a somatic cell and molecular genetic laboratory for pursuit of research concerning the development of experimental models to study how human genes are regulated and, more importantly, to assess the roles of these regulatory genes in aging. For the coming year, we plan to continue with these studies and, specifically, our aims are to: (a) investigate our hypothesis that transformabilities of human cells by viruses are influenced by the constitutiveness of the anti-viral gene. The extent to which this gene is constitutive appears to be correlated to age. This being so, we will develop a model to investigate any association between the deregulation of the anti-viral genes and the frequency of oncogenic diseases in the aged; (b) to investigate the possibility that one of the pleiotypic effects of interferon (now regarded for its role as a regulatory molecule in differentiated

tissues) is the recognition of altered messenger RNAs in aging cells; and (c) substantiate the existence of regulatory gene elements in human cells by somatic cell genetics and somatic cell hybridization. In addition, we plan to ascertain whether the antitumor gene(s) and anti-viral genes are two separate genes or the same genes which mediate its action through a pleiotypic gene product.

Honors and Awards:

Dr. Tan was appointed Assistant Professor, Department of Pediatrics, Johns Hopkins University.

Dr. Tan was invited to chair "Anti-tumor assays of human interferon" at the International Workshop on Interferon in the treatment of cancer, sponsored by NCI and Memorial Sloan-Kettering Cancer Center.

Publications:

Tan, Y. H.: Chromosome-21-dosage effect on inducibility of anti-viral gene(s). Nature 253: (5489) 280-282, 1975.

Tan, Y. H.: Cell Fusion: an instrument for gene analysis in man. La Recherche. In press.

Tan, Y. H., Chou, E. L. and Lundh, N.: The regulation of chromosome 21 directed antiviral gene(s) as a consequence of age. Nature. In press.

Serial No.: ZO1-AG-00085-03-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Diet Probes to Study Aging Immunologic and Biochemical Functions

Previous Serial Number: HD-CCP-14

Principal Investigator: Gordon Stoltzner

Other Investigators: Takashi Makinodan

Cooperating Units: None

Man Years:

Total:	1.45
Professional:	0.55
Others:	0.9

Project Description:

Objectives: Over thirty years ago, it was discovered that moderately severe caloric restriction in developing rats results in significantly prolonged lifespan, up to 50% greater than normally fed control animals. It is the purpose of this study to measure the effects of five dietary regimens maintained in mice from the time of weaning as they age. In addition to analyses of longevity and body weight, the investigation will correlate a variety of immunologic and biochemical indices among the dietary groups.

Methods Employed: The five dietary groups utilized in this study are: (1) 24% protein from weaning until death or sacrifice; (2) 24% protein from weaning until 128 days, then switch to 8% protein diet until death of sacrifice; (3) 24% protein from weaning until 128 days, then switch to 4% protein diet; (4) 8% protein diet from weaning (3 weeks); and (5) 4% protein diet from weaning.

Fifty (50) mice from each group of animals placed on the five dietary regimens have been set aside for longevity analysis. The remaining mice are being utilized for a variety of biochemical and immunologic assays that are standard in this laboratory and are now in progress. These studies include: (1) weekly body weights; (2) daily assessment of mortality; (3) monthly dermatitis index; (4) sacrificial biochemical studies of (a) protein determinations on liver cytosols and kidney homogenates, (b) kidney catalase determination, and (c) liver aldolase determinations;

(5) sacrificial immunologic studies of (a) splenic lymphocyte proliferative responses to three mitogens and allogenic tissue, and (b) primary SRBC antibody and plaque response; (6) other studies of (a) organ weights, (b) selected histologic studies, and (c) hematocrit, peripheral white blood count, serum protein.

The sacrifice schedule is as follows: 12 months, 3/19-4/6/75; 18 months, 4/14-4/30/75; 4 months, 4/20-5/5/75; 24 months, 9/1-9/20/75; and more than 24 months, after 10/75.

Major Findings: In terms of calendar time, the diet study is now almost 70% completed. But from the labor standpoint, at least 50% of the work lies ahead.

The carefully executed body weight determinations have clearly demonstrated differences between the control animals, the two switch groups, the straight 8% and the straight 4% animals. The weight range is 34 grams for the control and 25 grams for the 4% group.

The mice are now nearing the 50% survival point. The two straight protein restriction groups (4 and 8%) have the lowest survival.

Experimental data from the 12 month sacrifice are being gathered at the time of this writing, and will be analyzed over the next few weeks.

Significance to Biomedical Research and the Program of the Institute: Dietary manipulation is a very important if not the only method for prolonging normal life expectancy in certain mammals. This study, utilizing a rather comprehensive experimental design, shall define the biochemical and immunologic effects of protein restriction in mice, and by the nature of these studies provide insight into mechanisms of these effects. Additionally, it will correlate the experimental findings with morphologic and longevity analyses.

Proposed Course: The experimental protocol dictates a sequential series of analyses as outlined above, so that the study will be finished during the autumn of 1975. Since much of the data are presently being gathered, no definite formulations regarding further experimental work in this area have been made.

Keyword Descriptors: Aging, dietary restriction, immunology, enzymes

Honors and Awards: None

Publications: None

Serial No.: ZO1-AG-00086-01-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Rat as a Model for the Immunologic Study of Aging

Previous Serial Number: None

Principal Investigator: Gordon Stoltzner

Other Investigators: Takashi Makinodan

Cooperating Units: None

Man Years:

Total:	0.85
Professional:	0.4
Others:	0.45

Project Description:

Objectives: The rat is a typical mammal that is senescent by three years of age. It is the purpose of this project to carefully document declining function in the compartments of the immunologic system as measured by several standard indices. Of particular interest is comparing the function of blood lymphocytes with other aspects of the immunologic system, since blood is a tissue that can be easily and repeatedly obtained over time from the same subject animal.

Methods Employed: Utilizing various aged (weanling to > 24 month) male Fisher 344 rats, spleens, thymuses, and blood lymphocytes are harvested and cultured for three days in the presence of phytohemagglutinin, pokeweed, conconavalin A or xenogeneic irradiated lymphocytes. Proliferative responses as measured by incorporation of ^{3}H-thymidine are obtained by standard procedures in the laboratory.

Major Findings: This work was initiated in September, 1974, and hopefully can be completed by the end of this summer. The work from its inception has been troubled with variability of culture results from one assay to the next. Attention to media type, serum type and preparation, mitogen concentration, cell concentration, removal of possible repressor cells by nylon wood columns, cell dispersion techniques, culture time, incubator temperature, incubator carbon dioxide tension and other factors have not provided an answer to this problem. Ongoing experiments will hopefully resolve this

problem of reproducibility, so that this project can be brought to its intended conclusion.

Nevertheless, several reliable rat studies by this investigator have demonstrated a profound decline in all immunologic tissue studied from aged Fisher rats when compared to younger animals.

Significance to Biomedical Research and the Program of the Institute: Most mammalian organ systems, including the immunologic one, have declining functional capabilities with age. Changes in the immunologic system are particularly important since diminished immunologic activity can be directly correlated with increased susceptibility to a variety of infectious agents as well as the dramatically increased incidence of neoplasia with age, both of which are principal causes of death.

The purpose of this project is to define the rat as a suitable model system for the study of immunologic aging. The rat is an ideal animal since it ages rapidly, is relatively inexpensive to keep, but has sufficient body mass to permit serial sampling of blood and other tissues over time.

Proposed Course: Presently, various studies as cited above are being performed so that the study will be finished during the summer of 1975. There remains the possibility of expanding this work to another line of rats, namely, the Wistar rat.

Keyword Descriptors: Aging, immunology, rat.

Honors and Awards: None.

Publications: None.

Serial No.: Z01-AG-00087-02-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mechanism of the Maternal Age Effect

Previous Serial Number: HD-CCP-26

Principal Investigator: Edward L. Schneider

Other Investigators: T. Makinodan

Cooperating Units: None

Man Years:

Total:	1.6
Professional:	0.5
Others:	1.1

Project Description:

Objectives: It has been clearly established that with increased maternal age there is a greatly increased risk of children being born with chromosomal disorders. Despite considerable speculation about the cause of this maternal age effect, research to delineate the mechanisms of this effect has been limited by the practical as well as ethical considerations of human experimentation. However, the laboratory mouse can be used as an animal model for this effect, since with increasing mouse maternal age, there is an augmented frequency of fetuses with chromosomal alterations.

Initial studies were directed at finding an inbred mouse strain with an increased frequency of maternal age-related, chromosomally abnormal offspring. Subsequent studies are directed at examining particular mechanisms, such as autoimmunity, which have been suggested to be responsible for the maternal age effect.

Methods Employed:
1. Young females and retired female breeders from five mouse inbred strains (A/J, C57B16, C3H, CBA, SJL) are mated with young males. Pregnant females are sacrificed between ten and thirteen gestational days. The embryos are removed, abnormal embryos are photographed, and chromosome preparations are made from all embryos. Currently, chromosomal banding techniques are being refined in order to individually identify all the mouse chromosomes. With this refinement, chromosomal analysis should detect fetuses with specific chromosomal alterations.

2. Autoimmunity is induced in female A/J mice by thymectomy at 4-5 weeks of age. At 6 to 8 months, blood samples are taken to measure antinuclear antibody titers. If the animals exhibit autoimmunity, as indicated by the presence of antinuclear antibodies, they are mated to young males and chromosomal preparations are made from their embryos as described above. Embryos from sham-operated and non-operated age-matched A/J mice are used as controls. At the time of sacrifice, sera is taken from each mother for measurement of antinuclear antibody titers.

Major Findings: Preliminary results indicate that with increased maternal age the frequency of chromosomally abnormal fetuses increases from 3% (in the 3-5 month old range) to 15% (in the 8 to 12 month old range). The most frequent forms of observed aneuploidy are mosiacism and trisomies. The maternal age effect appears to be present in all the mouse inbred strains examined.

A good correlation has been observed between morphologically abnormal fetuses and chromosomal abnormalities. With chromosomal banding, it should be possible to identify the chromosomal alteration responsible for specific patterns of morphological abnormalities.

Significance to Biomedical Research and the Program of the Institute: Chromosomal disorders are extraordinarily common in man with a frequency of approximately 1 in 100 live births. This frequency is even higher during gestation and results in spontaneous abortions. By the maternal age of 45, a woman may have a 5% risk of having a child with severe mental and physical retardation (Down's Syndrome). Even with the advent of prenatal diagnosis, children with this disorder will continue to be born and require a lifetime of specialized care.

Utilizing an animal model, such as the laboratory mouse, it is anticipated that insight may be gained into the mechanisms of the production of chromosomally abnormal fetuses. An understanding of these mechanisms could lead to the development of techniques to detect mothers at increased risk and lead to higher frequencies of successful prenatal chromosomal diagnosis.

In addition, these studies will contribute to our understanding of the aging of the female reproductive system.

Proposed Course: Studies of the effect of autoimmunity will be pursued to assess the impact of an altered immune environment on the frequency of chromosomally abnormal offspring. Further studies will be directed at delineating the etiologic role of radiation, infectious agents and uterine environment.

Keyword Descriptors: Maternal age, chromosomally abnormal offspring, mouse model, effect of autoimmunity.

Honors and Awards: None

Publications: None

Serial No.: Z01-AG-00088-03-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mechanisms of Cellular Aging

Previous Serial Number: HD-CCP-19

Principal Investigator: Edward L. Schneider

Other Investigators: Youji Mitsui

Man Years:

Total:	4.4
Professional:	1.6
Others:	2.8

Project Description:

Objectives: A decline in the replicative ability of certain cell populations is an important aspect of human aging. Because of the practical and ethical limitations of in vitro experimentation, considerable effort has been expended to create in vitro models for studying the mechanisms of this age-related decrease in cell replication. One frequently used model is the cultured human diploid lung fibroblast (WI-38). Initial studies are examining the temporal sequence of the cellular alterations that occur as these cultured human diploid cells progress through their in vitro lifespan. Particular attention is focused on the relation of these alterations to the decline in cell population replication.

To obtain an even more appropriate in vitro model for studying human cellular aging, skin fibroblast cultures were obtained from over 50 members of the Baltimore Longitudinal Study (ages 20-95). Cell population replication and several other parameters which are altered by in vitro cellular aging can therefore be measured in these cultures at the same early passage as a function of in vivo age.

Another important aspect of this project is to examine the relationship between chromosomal alterations (aneuploidy), aging and abnormal cell replication (malignancy). Initial studies will focus on the frequency of aneuploidy with increasing in vitro and in vivo cellular aging. Cell replication and aneuploidy will also be examined in cell cultures derived from patients with human genetic disorders, such as Gardner's syndrome, which feature a marked increased frequency of malignant tumors.

Methods Employed:
1. A cell bank has been established for skin fibroblast cultures derived from members of the Baltimore Longitudinal Study from patients with Gardner's

syndrome and Down's syndrome, and from age-matched siblings or spouses.

2. Cell population replication kinetics are determined by standard growth curve determination, measurement of percent radioisotope labeled nuclei after tritiated thymidine incubation, and by cell cycle analysis with a flow microfluorometer.

3. Cell volume distribution is determined using a Coulter counter and channelizer. Measurement of cell volumes in cell populations in defined cell cycle phases is accomplished with a multiparameter flow microfluorometer.

4. Chromosomal studies include the measurement of the frequency of aneuploidy using standard karyotypic analysis and determination of baseline sister-chromatid exchanges using the differential chromatid staining resulting from BUdR incorporation into DNA.

Major Findings:

1. Cultured human diploid fibroblasts (WI-38 cells) were found to have a highly predictable sequence of cellular alterations as they progress through their in vitro lifespan. The initial alterations are a gradual decrease in maximal cell population replication rate and an equally slow shift to large cell volumes.

2. The association observed between cell volume and cell replication led to a series of experiments to delineate their relationship. These studies indicated that the cell volume alterations are most likely the consequence rather than the cause of the decreased cell replication observed as cells "age" in vitro. Multiparameter flow microfluorometric analysis indicates that the increase in cell volumes observed in senescent cells involves cells in all phases of the cell cycle.

3. Cells from patients with Gardner's syndrome, a premalignant genetic disorder, revealed a marked increased frequency of (chromosomal) aneuploidy when compared with cells from age-matched controls [24.4% (G.S.) vs 9.2% (Cont)]. Baseline sister-chromatid exchange and repair of mithramycin-C induced chromosomal breakage appear to be unaltered in these cells.

Significance to Biomedical Research and the Program of the Institute: Examination of the sequence of cellular alterations that occur as cultured human diploid cells progress through their in vitro lifespan emphasizes the vital role of the early decline in cell population replication. Flow microfluorometric studies indicate that there is an increased proportion of cells arrested in the G_1 cell cycle phase in "senescent" cell cultures. Isolation of these non-dividing or slowly dividing G_1 cells may provide an opportunity to convert them to rapidly dividing cells. Conversion of non-dividing or slowly dividing cells to rapidly dividing cells in vitro would be an important step in learning how to rejuvenate aged non-dividing and slowly dividing cells in vivo.

Current studies are focused on skin fibroblast cultures derived from old and young adult members of the Baltimore Longitudinal Study. It will be of interest to discern whether the cellular alterations observed in fibroblasts as a function of in vitro age also occur as a function of in vivo age.

The finding of increased chromosomal aneuploidy in cells from individuals with a premalignant genetic disorder (Gardner's syndrome) raises several

intriguing questions: (i) is aneuploidy the primary genetic abnormality and malignancy the consequence of chromosomal imbalance, (ii) does the single gene product result in both aneuploidy and malignancy or is aneuploidy merely the result of the pre-neoplastic process?

Proposed Course: Cell population replication, chromosome complement, volume distribution, macromolecule content, RNA metabolism, and repair of chromosomal damage will be examined in cell cultures derived from young and old adult donors (members of the Baltimore Longitudinal Study).

Since non-replicating cells appear to have larger volumes and greater densities, attempts will be made to separate out discreet populations of replicating and non-replicating cells by isopycnic banding, velocity sedimentation, and/or cell sorting (with a multiparameter cell sorter).

Members of Gardner syndrome families, particularly younger individuals, will be studied to discern whether the observed aneuploidy precedes or follows tumor development. Cell cultures derived from these individuals will be examined for cell replication capability, susceptibility to SV_{40} transformation, and ability to repair x-ray and UV induced DNA damage.

Honors and Awards:

Dr. Schneider presented seminars related to this work at Stanford University, Johns Hopkins Hospital, the University of Maryland, and Cornell University School of Medicine.

Dr. Schneider participated in the Fourth Annual Workshop on the Biology of Aging at the Scripps Institution of Oceanography, March 24-26, 1975; and was invited to participate in a symposium entitled "Aging of Cells in Culture" at the 10th International Congress of Gerontology, June 22-27, 1975, in Jerusalem, Israel.

Publications:

Schneider, E.L. and Chase, G.: Relationship between age of donor and *in vitro* lifespan of human diploid fibroblasts. *Gerontologia*. In press.

Tice, R. and Schneider, E.L.: *In vitro* aspects of human genetic disorders which reduce accelerated aging. *Gerontologia*. In press.

Schneider, E.L. and Mitsui, Y.: Temporal sequence of "aging" parameters in cultured human diploid fibroblasts. *10th International Congress of Gerontology*. In press.

Schneider, E. and Stanbridge, E.: Comparison of methods for the detection of mycoplasma contamination of cell cultures: a review. *In Vitro*. In press.

Schneider, E.: Detection of Mycoplasma Contamination in Cultured Cells: Comparison of Biochemical, Morphological and Microbiological Techniques. In *Methods in Cell Biology*. In press.

Schneider, E. and Stanbridge, E.: A Simple Biochemical Technique for the Detection of Mycoplasma Contamination of Cultured Cells. In *Methods in Cell Biology*. In press.

Serial No.: Z01-AG-00089-01-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Human Cross-Sectional and Longitudinal Immunology Program

Previous Serial Number: None

Principal Investigator: Marguerite M. B. Kay

Other Investigators: T. Makinodan
K. Nakamura EOD 4/75

Cooperating Units: Department of Epidemiology
University of Maryland
Baltimore, Maryland

Man Years:

Total:	0.4
Professional:	0.3
Others:	0.1

Project Description:

Objectives: A decline of cellular immunity with age has been documented in humans, guinea pigs, and mice. MacKay has shown a direct correlation between integrity of the immune system and longevity in humans. The number of white cells in the blood does not change significantly after sexual maturity although the number of circulating T cells in humans has been variously reported to: decline progressively, rise, or remain the same with age. The level of circulating immunoglobulins (Ig) tends to increase slightly with age. Primary, but not secondary, antibody response decreases with age. A decline in antibody response has been reported to occur as early as the beginning of thymic involution. This would suggest that with many types of primary antibody responses, aging is affecting the regulatory T cells and not necessarily the B cells. At the present time, because of lack of investigation and/or conflicting reports in the literature, it is not known whether the number of T cells decreases with increasing age or whether T cells in humans become functionally less effective with age. Studies in mice indicate that the latter is the case, but have not suggested the mechanism(s) responsible for the decline in responsiveness at the cellular level. The objective of this project is to determine (a) the nature of the decline and (b) the mechanisms responsible.

Methods Employed: Both T and B cell functions will be assayed by immunofluorescence, SRBC rosettes, response to mitogens and antigens, and skin tests.

Major Findings:

1. The cells found attached to Reed-Sternberg cells in the lymph nodes of patients with Hodgkin's disease have been demonstrated to be T cell.

2. The Reed-Sternberg cells do not have any of the surface characteristics of T cells. By surface morphology, they appear to be macrophages. It was found that in patients with Hodgkin's disease "killer" T cells cytolyze autologous neoplastic Reed-Sternberg cells in the following sequential manner: stage 1, T cells affix the tips of their microvilli onto target cells; stage 2, T cells subject target cell membranes to shearing and tearing forces which produce gaps and holes; stage 3, target cells lyse and T cells "crawl" away.

3. It has been determined that some transient autoimmune hemolytic anemias following respiratory infections are accompanied by depressed T cell responsiveness.

Proposed Course: As outlined above. Project just initiated.

Keyword Descriptors: Human longitudinal immunology, cellular immunity, decline in immunocompetence, longevity, regulatory T cells, antibody response, mechanisms of decreased responsiveness.

Honors and Awards:

Dr. Kay was invited to participate in a symposium entitled "Immune Problems Late in Life," sponsored by the American Geriatrics Society. She discussed autoimmune problems of the aged.

Publications:

Kay, M.M.B. and Kadin, M.: Classification of Hodgkin's cells according to surface characteristics. Lancet 1, 7909: 748-749, 1975.

Serial No.: Z01-AG-00090-01-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Membrane Differentiation: Effect of Aging on Modulation of Receptor Formation

Previous Serial Number: None

Principal Investigator: Marguerite M. B. Kay

Other Investigators: T. Makinodan

Cooperating Units: None

Man Years:

Total:	1.38
Professional:	0.58
Others:	0.8

Project Description:

Objectives: Immunologic activities generally decline as an individual ages. This appears to be due to a decline in the proliferation and differentiation of antigen sensitive precursor cells. Since the early events in antigen activation occur at the level of the cell membrane, the effect of aging on membrane receptors and function will be investigated.

Methods Employed: Splenic cells from young and old mice were separated into subpopulations by their differences in density, adherence to nylon wool, and differential susceptibility to antisera reagents. The subpopulations were viewed with scanning electron microscopy following each treatment. Human lymphocytes were separated into T and B cell populations using the SRBC rosette technique followed by density separation on Ficoll/Hypaque. Subpopulations of B lymphocytes were identified by the use of antisera reagents (anti-human IgA, M or G) conjugated to scanning electron microscopy visible markers.

Major Findings:
1. The lymphoid cells of old mice are more fragile under conditions where antisera is employed than are the cells from young mice.

2. A new tri-labelling technique was developed which permits unambiguous identification of subpopulations of cells and quantitation of distinct cell surface receptors on individual cells as well as permitting kinetic studies of the membrane changes following the binding of a liquid with its receptor.

Significance to Biomedical Research and the Program of the Institute: The results of these studies are vital to our understanding of aging at a cellular level, and will form the basis of studies directed toward reconstitution of immune function in the aged.

Proposed Course; The newly developed tri-labelling technique will be applied to membrane studies of cells from young and old mice and humans in an attempt to characterize the membrane defects associated with aging.

Keyword Descriptors: Decreased immune activities, proliferation and differentiation of antigen sensitive precursor cells, scanning electron microscopy (SEM), increased fragility of old lymphocytes, tri-labelling technique for SEM, kinetic studies, modulation of receptor formation

Honors and Awards:

Dr. Kay served as organizer and chairman of the Annual Educational Symposium of the Biological Science Section at the 27th Annual Meeting of the Gerontological Society on October 31, 1974, in Portland, Oregon. The invited speakers at the "Biology of Aging: Immunobiology" Symposium were Drs. Robert Auerbach (University of Wisconsin), H. Hugh Fudenberg (University of California, San Francisco and University of South Carolina), and Eli Sercarz (University of California, Los Angeles).

Dr. Kay was invited to participate in a symposium entitled "Immunity and Aging" at the 10th International Congress of Gerontology, June 22-27, 1975, in Jerusalem, Israel.

Dr. Kay has been invited to serve as (a) the biological consultant to Dr. P. Lin, Supervisor, Users Electron Microscopy Laboratory, Enrico Fermi Institute, University of Chicago, Chicago, Illinois; (b) Electron Microscopy consultant to the Department of Basic and Clinical Immunology and Microbiology (Chairman: H. H. Fudenberg), Medical University of South Carolina, Charleston, South Carolina; and (c) Immunological consultant to Tago, Inc., Immunodiagnostic Reagents, So. San Francisco, California.

Publications:

Kay, M.M.B.: Multiple labeling technique used for kinetic studies of activated human B lymphocytes. Nature 254: 424-426, 1975.

Serial No.: Z01-AG-00091-01-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mechanism of In Situ Removal of Senescent Cells by Macrophages

Previous Serial Number: None

Principal Investigator: Marguerite M. B. Kay

Other Investigators: None

Cooperating Units: Dr. Paul Lin
The Users Electron Microscopy Laboratory
Enrico Fermi Institute
University of Chicago
Chicago, Illinois

Man Years:

Total:	0.9
Professional:	0.4
Others:	0.5

Project Description:

Objectives: Since macrophages phagocytize senescent self cells, they must be capable of distinguishing between "adult self" and "senescent self" cells. Therefore, studies were initiated to investigate and elucidate the mechanism by which macrophages recognize "senescent-self" cells. It was hypothesized that immunoglobulin (Ig) in normal human serum attach to the surface of senescent RBC and that the quantity of attached Ig increases with increasing age of RBC until a critical level is reached at that time macrophages no longer can recognize these RBC as "self" cells.

Methods Employed: In this investigation, an attempt was made to adhere as closely as possible to in situ conditions using short term culture techniques. Accordingly, human macrophages, isolated on "Lymphoprep" followed by glass adherence, were incubated with the individual's own RBC and own serum Ig, rather than a specific but foreign anti-RBC reagent. In vitro aged RBC were obtained by storage in serum-free medium. In situ aged RBC were obtained by density separation according to the method of Murphy. Autologous Ig was prepared by fractionation of whole serum with $(NH_4)_2 SO_4$, autologous IgG by ion exchange chromatography, Ig depleted serum by dialysis of the serum remaining after salt fractionation. Electron microscopy markers were conjugated to anti-human IgA, M or G globulin as described in PHS-NIH individual

project report Z01-AG-00090-01-LCP. Neuraminidase was prepared by affinity chromatography by the method of Cuatrecasas.

Major Findings:

1. A new method for quantitating phagocytosis was developed. RBC were counted (t_0), added to test tubes containing macrophages, incubated for 3 hours at 37°C, agitated vigorously with a "Vortex" mixer and counted again in a hemocytometer or Coulter counter (t_3). The percent phagocytosis was determined according to the following equation:

$$\left[\frac{RBC_{(t_0)} - RBC_{(t_3)}}{RBC_{(t_0)}}\right] \times 100 = \% \text{ phagocytosis}$$

This equation is valid so long as $RBC_{(t_3)}$ is reasonably small relative to $RBC_{(t_0)}$. This was generally the case under the conditions employed in these experiments. This assay has the advantage of quantitating macrophage activity and fate of large numbers of RBC quickly as well as cheaply.

2. It was proven both by indirect and direct methods that macrophages can differentiate "adult self" from "senescent self" on the basis of selective IgG attachment to the surface of senescent cells.

3. It was shown that IgG bound to young RBC pretreated with Vibrio cholerae neuraminidase. These "artificially" aged cells were phagocytized as readily as in situ aged RBC suggesting exposure of carbohydrate moieties may occur with increasing in situ age thus initiating IgG binding.

Significance to Biomedical Research and the Program of the Institute: The results of these experiments are vital to our understanding of the mechanisms by which senescent cells are removed and suggest that receptor molecules on cell membranes "age."

Proposed Course: Studies will focus on molecular characterization of aging of terminally differentiated cells, and effete cell-macrophage interactions.

Keyword Descriptors: Macrophage, phagocytosis of senescent self cells, selective immunoglobulin attachment, new method of quantitating phagocytosis, scanning electron microscopy detection of IgG, "artificial" aging of young RBC by neuraminidase

Honors and Awards:

Dr. Kay presented part of this work at the 27th Annual Meeting of the Gerontological Society in Portland, Oregon, on October 30, 1974.

Publications: None

Serial No.: Z01-AG-00092-03-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cellular Aspects of the Immune Response of Long-term Allogeneic Chimeras

Previous Serial Number: HD-CCP-15

Principal Investigator: Albert A. Nordin

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: The major objective of the proposed research is to examine the immune system of x-irradiated allogeneic bone marrow grafted mice. These allogeneic radiation chimeras serve as a model for investigating the basic cellular aspects of immune mechanisms with particular emphasis on regulatory mechanisms involved in immunologic responsiveness.

Methods Employed: Previous work has established the fact that allogeneic bone marrow chimeric mice can only survive the secondary disease effects of a graft-versus-host (GVH) reaction in a controlled germfree environment. However, there is reasonable evidence to suggest that the clean environment provided by a laminar flow animal facility will also promote long-term survival. Animals quartered in a laminar flow room are given a low lethal dose of x-irradiation followed by a bone marrow graft from either a syngeneic or allogeneic donor. Subsequently observed immunologic deficiency will be examined to determine the cellular aspects of the deficiency. Experimental attempts to compensate for or eliminate the deficiency will be carried out.

Major Findings:
1. Allogeneic radiation chimeras established and maintained in a laminar flow environment for 8-10 months post-bone marrow transplant are by several immunological criteria different from similar chimeras maintained in the

germfree environment. The main difference is that the H-2 type of lymphocytes were all donor derived during the entire lifespan of the chimeras. However, the H-2 type of the lymphocytes of the chimeras maintained in the laminar flow environment for 8-10 months was either host, donor or mixtures of both.

2. In mixed lymphocyte cultures, it is apparent that splenic lymphocytes of allogeneic chimeras react to the opposite H-2 type. For example, in C3H/He mice ($H\text{-}2^k$) which were irradiated and transplanted with DBA/2 ($H\text{-}2^d$) bone marrow, mice which have $H\text{-}2^k$ lymphocytes reacted as if they had previously been immunized to $H\text{-}2^d$ cells.

Significance to Biomedical Research and the Program of the Institute: Bone marrow grafting within a heterogeneous population has necessarily been restricted, mainly as a result of immunologic damage to the host. Techniques are available to experimentally determine not only the favorable conditions which permit the establishing of allogeneic bone marrow chimeric mice but, also, to define the events resulting in immunoregulation observed in such animals. Information may then be available which will reduce the risks in bone marrow grafting and result in the reconstitution of an intact immune mechanism.

Cellular immunology during recent years revolved around cell cooperation as a basic requisite for the expression of antibody to many antigens. Although the majority of such studies have considered the interactions between thymus dependent and bone marrow dependent cells, it is becoming increasingly obvious that other cell type cooperative efforts are involved in the complex immune systems of mammals. The deficiency of any one cell type most likely results in regulating the expression of immunity involving several lymphoid elements. The information which can be supplied in attempts to re-establish immunological competence to the allogeneic chimeric mice is expected to demonstrate and define the importance of cellular interactions in both the humoral and cell-mediated forms of the immune response.

Proposed Course:

1. To establish the technique of testing the H-2 type of circulating mouse lymphocytes as a means of determining the chimeric state.

2. To then continue the mixed lymphocyte reaction experiments in an attempt to distinguish if chimeric mice contain reactive clones, as indicated by our data, which is in conflict to some other reported data that indicates a tolerant state exists in chimeric mice (J.E.M. 141, 322, 1975).

3. To examine the cell-mediated immune potential of spleen cells of chimeric mice using *in vitro* techniques.

4. To transplant thymus grafts of either host or donor type (depending on H-2 type of lymphocytes) in an attempt to restore immunodeficiency without precipitating a graft-versus-host reaction.

Keyword Descriptors: Allogeneic bone marrow chimera, thymic deficiency, Graft-versus-Host, cell-mediated immunity, tolerance

Honors and Awards: None

Publications: None

Serial No.: ZO1-AG-00093-03-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cellular Basis of Regulation of the Humoral Immune Response

Previous Serial Number: HD-CCP-14

Principal Investigator: Albert A. Nordin

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	0.7
Others:	0.8

Project Description:

Objectives: The goal of this project is to characterize the regulation of the immune response by cellular elements. Efforts to determine the origin and mechanism of action of these cells are of prime interest.

Methods Employed:

1. B-cell source. Young C57Bl/6 mice (6 weeks of age) are thymectomized, irradiated 2 weeks later and grafted with 10×10^6 syngeneic bone marrow cells 24 hours after irradiation. Four to eight weeks later, the spleens of these mice serve as a source of bone marrow derived lymphocytes (B-cells).

2. T-cell source. Six to eight week old C57Bl/6 or $B_6D_2F_1$ mice are used as donors for thymus glands. X-irradiated (850r) syngeneic mice are injected with $50\text{-}70 \times 10^6$ thymus cells with or without a simultaneous injection of antigen. Seven or eight days later, the spleens of these mice are used as a source of thymus derived lymphocytes (T-cells). Those cells from mice injected with thymocytes and antigen are referred to as activated T-cells and those from mice receiving only thymocytes are referred to as normal T-cells.

3. Adherent cells from carbonyl iron treated spleen. After treating spleen cells with carbonyl iron, the remaining population is placed in plastic dishes and incubated for one hour. Nonadherent cells are removed and the adherent layer washed exhaustively. The adherent cells are removed by trypsin in a viable state.

4. The in vitro culture technique and the assay for plaque-forming cells are routine methods.

Major Findings:

1. A naturally occurring cellular component of the spleen, capable of regulating the in vitro immune response to sheep erythrocytes, has been demonstrated.

   a. This population of the cells has been shown to be lymphocytic by morphological criteria by both light and electron microscopy.
   b. These regulatory lymphocytes respond to LPS but not to PHA or Con-A. The response to LPS is resistant to anti-θ and C' treatment.
   c. By immunofluorescence, 95 % of these lymphocytes express membrane bound immunoglobulin.
   d. The in vitro response to a T-independent antigen, DAGG-Ficoll, is not affected by the addition of these lymphocytic elements.

2. Spleen cells from aging C57B1/6 mice (20-24 months old), when studied individually using in vitro techniques, showed many patterns of immunodeficiencies. Even though the mice were highly inbred, there was no consistent pattern of immunosenescence; i.e., some showed T-helper cell deficiencies, others B-cell deficiencies and others T-killer cell deficiencies. It was also significant that the results of one assay could not predict the results of another assay; i.e., (1) T-helper cell function does not correlate with T-killer cell actively, and (2) functional B-cells, as indicated by a good PFC response to sheep erythrocytes, do not correlate with the response to DAGG-Ficoll. Finally, the response to mitogens did not correlate with any functional T or B cell activity.

Significance to Biomedical Research and the Program of the Institute: The goal of this research program is to examine the cellular populations that are regulating the humoral immune response. The mechanisms by which the regulation takes place would be of significance not only to the field of immunology but would have relevance to cell biology. It is also significant to the area of immunosenescence. The decline in immunological responsiveness with age is well established but the reasons are not at all understood. The role of regulatory mechanisms in explaining the phenomena of immunosenescence may be of considerable significance.

Proposed Course:

1. To continue in an attempt to further purify and characterize the nature of the regulatory lymphocytes.

2. To determine the effect of regulatory lymphocytes on the cell-mediated immune response.

3. To continue the investigations on old mice in an attempt to characterize the nature of the subtle and various immunodeficiencies observed as well as the mechanisms underlying the induction of the deficiencies.

Keyword Descriptors: Immunoregulation, antibody formation, *in vitro*, cell separation, regulatory lymphocyte population, immunosenescence

Honors and Awards:

Dr. Albert A. Nordin was a member of the Council of the Regulatory Biology Section of the National Science Foundation through March, 1975.

Publications:

Nordin, A.A. and Makinodan, T.: Humoral immunity in aging. *Fed. Proc.* 33: 2033-2035, 1974.

Seibert, K., Pollard, M. and Nordin, A.: Some aspects of humoral immunity in germ-free and conventional SJL/J mice in relation to age and pathology. *Cancer Research* 34: (7), 1707-1719, July, 1974.

Serial No.: Z01-AG-00094-02-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Characterization of Immune System of Aging Mice with Immunodeficiency Diseases

Previous Serial Number: HD-CCP-27

Principal Investigator: Toshio Hirano

Other Investigators: Albert A. Nordin

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	1.2
Others:	0.1

Project Description:

Objectives: The goal of this project is to characterize the immune system in young and aging mice of various genetic strains that show a high frequency of immunodeficiency disease. The relationship between the immunological disorders and the regulation, in the form of suppression, of the immune system will be investigated.

Methods Employed:
1. A modification of the spleen cell culture system of Mishel and Dutton is used. Spleen cells from individual mice or pooled spleen cells are cultured with mitomycin-C treated or irradiated allogeneic spleen cells, F_1 spleen cells or heterologous erythrocytes at 37° C for various days. In some cases, the Marbrook culture system is used.

2. Cytotoxicity assay - ^{51}Cr labeled EL-4 or P-815 cells are mixed with cultured spleen cells and incubated for various times. After incubation, cold PBS is added, the tubes centrifuged and the radioactivity of the supernatant counted.

3. Plaque-forming cell assay - routine technique used to detect IgM and IgG antibody-producing cells.

4. Direct Coomb's test is a routine method.

5. Thymidine Hot Pulse - high specific ^{3}H-thymidine (50 Ci/nM) was used in order to determine the proliferative phase.

6. Indirect Immunofluorescent technique - routine technique was used to detect the θ-positive cells.

Major Findings:

1. The development of cytotoxic lymphocytes (CL) decreases in aged NZB, DBA/2 and C57Bl/6 mice.

2. This decline in cell-mediated cytotoxicity is not correlated with the increased frequency of Coomb's positive reactions in aged NZB mice nor with a change in the frequency of θ-positive cells in spleen of NZB and DBA/2 mice.

3. Under the Con-A stimulation, spleen cells can differentiate to suppressor cells. Con-A-stimulated suppressor cells are anti-θ sensitive and cortisone sensitive.

4. The development of Con-A-stimulated suppressor cells decreases with age.

5. Under the stimulation of alloantigen, CL develop and at the same time natural suppressor cells also develop in mixed lymphocyte cultures.

6. Natural suppressor cells are anti-θ sensitive and cortisone sensitive. Since CL develop from cortisone pretreated spleen cells, natural suppressor cells and CL are most likely different T-subpopulation.

7. During the early life (1 week-5 weeks old), some abnormal or immature cell-mediated immune responses are seen in C57Bl/6 and NZB mice.

Significance to Biomedical Research and the Program of the Institute: This proposal offers two main significant contributions: (1) the regulatory mechanism of cell-mediated immunity in young and aged mice, and (2) the mechanism of the immunosenescence of cell-mediated immunity.

Proposed Course:

1. The regulatory mechanism in cell-mediated immune response in young and aged mice will be established.

2. The relationship between the abnormal immune response and the autoimmune disease will be characterized in order to investigate the mechanism of autoimmune disease.

Keyword Descriptors: Cell-mediated immunity, *in vitro*, suppressor cells, cytotoxic lymphocytes, immunodeficiency in aging.

Honors and Awards: None

Publications: None

Serial No.: Z01-AG-00095-02-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Role of Cell Membrane Structures on Cellular Recognition Phenomena

Previous Serial Number: HD-CCP-25

Principal Investigator: W. H. Adler

Other Investigators: J. W. Heine
M. A. Brock
K. Jones EOD 9/1/74
H. Nariuchi EOD 9/1/74

Cooperating Units: Carnegie Institute of Washington
Baltimore, Maryland

Man Years:

Total:	4.0
Professional:	3.2
Others:	0.8

Project Description:

Objectives: The project will attempt to correlate immunologic function with morphologically distinct cellular populations. Also, mice of different ages and strains will be studied to determine their ability to withstand an oncogenic stimulus, to reject syngeneic tumor cells, to be immunized to syngeneic tumor cells and to develop cytotoxic "T" lymphocytes which can kill allogeneic or syngeneic tumor cells in vitro and in vivo.

Methods Employed: The basis of the function assays are an in vitro lymphocyte culture system and in vivo cell transfer schema. The cells studied will be obtained from different tissues and from animals with altered immune capacity. Animals of varying ages as well as varying immune status will be examined to determine the basis of the alterations in functional capacity. Standard methods for the production of antisera will be used. The effects of the antisera will be assayed in the standard models available. Both in vitro cell killing techniques utilizing chromium^{51} release and growth inhibition, and in vivo transplantation techniques will be used. The analysis of cell types responsible for immunity of resistance will be determined by appropriate antisera.

Major Findings: The effects of x-irradiation on lymphoid cells demonstrate no inherent sensitivity or resistance of any particular lymphoid cell type. X-irradiation damage appears to act on a dose related absolute number of cells, and therefore the cell types which have a greater representation in murine lymphoid tissue are less effected since the damaged cells constitute a lesser percentage of the whole.

The use of soluble membrane antigen preparations, and selective techniques to kill lymphoid cells while maintaining their cellular membrane structure, has demonstrated that the lymphocyte "recognition" of cell allo-antigen in a mixed lymphocyte culture (MLC) is, in actuality, an interaction between the allogeneic cells and is not dependent on the antigenic recognition per se. What this means is that the target cell in the MLC is equally as important as the reactor cell. This finding explains why the cells from an immunologically deficient animal which respond poorly or not at all in most immune assays will respond in an MLC.

It was found that endotoxin (LPS), although a mitogenic probe for a sub-set of "B" lymphocytes, has as its target cell population a lymphoid cell which is undergoing division cycles early in the culture period. In most situations, these are "B" cells but, if "T" cells can meet these requirements, then they also can be stimulated by LPS.

Results on the role of exogenous cyclo-nucleotides in lymphocyte activation have demonstrated no positive correlation for either cyclic-AMP or cyclic-GMP. What has been shown, however, is that cyclic-AMP can stabilize lymphocyte and tumor cell membranes and, as such, can alter the ability to destroy these cells by immunologic means.

An antisera has been found which will selectively kill antibody-forming cell precursors while having no effect on cells making antibody. It has been determined that there is a marked age-related difference in resistance to the challenge of syngeneic methyl cholanthrene sarcoma cells.

Significance to Biomedical Research and the Program of the Institute: In order to understand what the meaning or effects of an immunodeficiency in aging animals are, we are going to have to better understand what entails normal immune function on a cellular level. It is proposed that research on the above lines will augment our basic appreciation of immunobiology, and with this knowledge, we will be able to diagnose and treat immunodeficiency.

Proposed Course: To continue to outline the connections between form and function and to expand our technical ability to measure function. We hope to develop better diagnostic criteria and tests to evaluate immune capacity.

Keyword Descriptors: Tumor immunity, subclasses of lymphocytes, in vitro lymphocyte proliferation, x-irradiation, "T" and "B" cells, age effects on immunity

Honors and Awards:

Seminars: Mitogenic Reactivity of Lymphocytes, Toronto Childrens Hospital, 10/3/74; The Mitogenic Activity of Endotoxin LPS, Queens University, Kingston, Ontario, 10/5/74; Aging and Immune Function, Jewish General Hospital, Montreal, Quebec, 10/6/74; LPS Activation of "T" Cells, Wayne State University, Detroit, Michigan, 11/6/74; The Effects of X-Irradiation of Lymphoid Cells, NIH-NIDR, 3/6/74.

Publications:

Ozato, K., Adler, E. H. and Ebert, J. D.: Triggering effects of LPS on con A stimulated mouse thymic lymphocytes. Cell. Immunol. In press.

Adler, W. H.: Aging and immune function. Bioscience. In press.

Ozato, K., Ebert, J. D. and Adler, W. H.: Pretreatment of thymocytes by PHA inhibits bonding of H^3-concanavalin A. J. Immunol. In press.

Adler, W. H.: An Autoimmune Theory of Aging. In Rockstein, M. (Ed.): Theoretical Aspects of Aging. Academic Press, New York, 1974, pp. 33-42.

Serial No.: Z01-AG-00096-02-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Low Temperature Effects on Cells of Aging Individuals

Previous Serial Number: HD-CCP-23

Principal Investigator: Mary Anne Brock

Other Investigators: W. H. Adler

Cooperating Units: None

Man Years:

Total	1.0
Professional:	0.9
Others:	0.1

Project Description:

Objectives: To define the age-related functional and structural characteristics of pre- and post-mitotic cell types and, specifically, (1) to explore possible age-related differences in the response of human and murine lymphohemopoietic cells to agents which affect the in vitro stimulatory action of mitogens; and (2) to investigate the possible age-related differences in biomembrane systems of lymphohemopoietic cells reflected in their differential susceptibility to freezing damage and ability to recover functionally after thawing.

Methods Employed: The functional capacity of mouse splenic cell suspensions was assessed from the rate of DNA synthesis as monitored by H^3-thymidine incorporation in vitro, with and without stimulation by the T-cell mitogens, PHA and Concanavalin A, and the B-cell mitogen, LPS. Several agents, as noted in the next section, were added to the cultures with H^3-thymidine.

Major Findings: Dose-response curves were determined for each of the mitogens with concentrations of H^3-thymidine systematically increased to 40 times the established dosage per culture. A marked enhancement of the response of splenic lymphocytes to each of the mitogens was observed and was directly related to increased concentration of labelled thymidine up to 5 μC and 8.3×10^{-4} μM thymidine per culture. At higher concentrations, the increase in the response was diminished but not reduced to control levels. Because the thymidine solution also contained very low concentrations of ethanol, the enhancement of the response to mitogens could be due to higher concentrations of thymidine, ethanol, or a combination of both. This effect could

be mediated directly on the plasma membrane or by augmentation of cellular metabolism by thymidine and/or ethanol resulting in increased DNA synthesis. Therefore, a variety of substrates and other molecular species were tested in the in vitro system. The glycolytic substrates, ribose-5-phosphate, inosine monophosphate and glucose-6-phosphate had little effect over that of the mitogens alone on lymphocytic stimulation except for a slight enhancement of the response to PHA or Con A (1.5 times) by inosine monophosphate and glucose-6-phosphate. Neither methanol nor mercaptoethanol had an appreciable effect on DNA synthesis. Cold thymidine, with or without increased labelled thymidine concentrations was generally inhibitory, probably due to dilution of the intracellular H^3-thymidine pool. Of all the agents tested, only ethanol with labelled thymidine in elevated concentrations showed consistency in enhancing the stimulatory action of the mitogens on splenic lymphocytes. If, however, ethanol was added with the mitogens at the beginning of the culture period, 2 days prior to the addition of labelled thymidine, the ethanol inhibited DNA synthesis.

Some caution should be employed in the interpretation of results reporting on DNA synthesis by splenic lymphocytes, because there appears to be a cyclic change in both the basal level of DNA synthesis and the stimulatory action of the mitogens. The peak to peak period has averaged about 26 days for 4 months in rooms where a 24-hour light-dark cycle is maintained. Combined with the periodic changes in basal DNA synthesis, the stimulatory action of the mitogens was modulated. This modulation could be interpreted either as a shift in the number of T and B lymphocytes in the cell population or as a change in the plasma membrane receptor sites on a constant or a variable number of T and B lymphocytes.

Because there have been unforeseen delays at the NIH Biomedical Engineering and Instrument Branch in the production of a sensitive and reliable instrument to control the cooling rate in freezing experiments, particularly the variability introduced at the point of freezing, we have attempted to modify the available Linde BF-4 Biological Freezing System. The changes to the system are in progress and include reduction in the rate of cooling to -0.1° C per minute. Neither a constant cooling rate nor the slow rate are attainable with the conventional Linde BF-4 System.

Significance to Biomedical Research and the Program of the Institute: The decline in the functional capacity of lymphocytes with age may be intrinsic and/or extrinsic. These possibilities can be tested by modifying components in an in vitro culture system that tests functional capacity and by assessing the effects of freeze-thaw damage on lymphocytic biomembrane systems. Controlled rate cooling techniques may be used to separate subpopulations of lymphocytes for further study at the cellular level.

Proposed Course: Major emphasis will be placed on the definition of possible age-related changes in the biomembrane systems of mammalian lymphoid cells. The action of ethanol and thymidine in enhancing the splenic lymphocytic response to mitogens will be further characterized and assessed in cell populations from older mice. Controlled rate freezing will be used to test age-related lymphocytic resistance to stress.

Keyword Descriptors: Aging, lymphohemopoietic cells, in vitro action of mitogens, DNA synthesis, constant cooling rate, freezing.

Honors and Awards:

Dr. Brock served as a member of the Board of Governors of the Society for Cryobiology.

Dr. Brock was invited to the Sixth International Interdisciplinary Cycle Research Symposium and the Seventh International Biometeorological Congress, College Park, Maryland, to be held August, 1975.

Publications;

Brock, M.A.: Growth, developmental and longevity rhythms in Campanularia flexuosa. Amer. Zool. 14: 757-771, 1974.

Brock, M.A.: Circannual rhythms I. Free-running rhythms in growth and development of the marine cnidarian, Campanularia flexuosa. Comp. Biochem. Physiol. In press.

Brock, M.A.: Circannual rhythms II. Temperature-compensated free-running rhythms in growth and development of the marine cnidarian, Campanularia flexuosa. Comp. Biochem. Physiol. In press.

Brock, M.A.: Circannual rhythms III. Rhythmicity in the longevity of hydranths of the marine cnidarian, Campanularia flexuosa. Comp. Biochem. Physiol. In press.

Brock, M.A.: Circannual rhythmicity in invertebrates. In Pengelley, E.T. (Ed.): Circannual Clocks, Annual Biological Rhythms. New York, Academic Press, 1974, pp 11-53.

Serial No.: Z01-AG-00097-01-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Use of Lectins and Other Molecular Probes to Study Cell Surface Receptors

Previous Serial Number: None

Principal Investigator: Joel H. Shaper

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.90
Professional:	0.65
Others:	0.25

Project Description:

Objectives: The ability to identify and correlate changes at the cell surface or membrane level as a consequence of cellular aging or of a specific biological insult such as viral transformation requires, as a prerequisite, a precise knowledge of the membrane structure. It is the purpose of this project to develop procedures whereby specific glycoprotein receptors and enzymatic activities which are intrinsic components of the plasma membrane can be identified (visualized by transmission or scanning electron microscopy) and isolated for subsequent physico-chemical characterization. The initial objectives of this project are to:

1. Visualize and determine the topographical distribution of the membrane bound Na^+-K^+ ATPase (Na^+-K^+ pump).

2. Isolate, characterize and compare unique lymphocyte plasma membrane glycoprotein receptors which bind mitogenic and nonmitogenic lectins possessing the same monosaccharide binding specificity.

3. Chemically produce a monovalent wheat germ lectin by the introduction of a sugar analogue covalently into the carbohydrate binding site, thereby correlating intrinsic valency of a lectin with biological activity.

Methods Employed: A ouabain analogue has been synthesized in which it is presumed that the intrinsic arabinose moiety has been converted into an N-substituted morpholine. The analogue is being chemically characterized and assessed for biological activity.

Procedures have been devised for the synthesis of bromacetyl derivatives of glucosamine. In addition, the synthesis of 2 , 3 epoxy-propyl-1-N-acetyl glucosamine by published procedures is underway. These glucosamine analogues will be tested for their ability to react covalently at the carbohydrate binding site of wheat germ agglutinin.

Lectins from wheat germ, lima bean and red kidney bean have been isolated and highly purified. These lectins will be insolubilized by conventional procedures to form affinity resins. Their ability to isolate detergent solubilized glycoprotein receptors will be investigated.

Major Findings: Projects have just been initiated; therefore, there are no major findings to date.

Significance to Biomedical Research and the Program of the Institute: Attention is currently focused on the thesis that alterations in cell membrane structure constituents are manifestations of changes in cellular regulatory mechanisms. Cell surface alterations are well documented in the case of viral transformation and conceivably may be diagnostic for events leading to the production of an "aging cell." The development of the methodologies to isolate and to study the localization and distribution of unique cell surface receptors and specific enzymatic activities can support or eliminate such a hypothesis.

Proposed Course: The analogues synthesized to date will be chemically characterized and assessed in their ability to serve as membrane markers for the Na^+-K^+ pump and affinity ligands for wheat germ agglutinin.

Keyword Descriptors: Visualization Na^+-K^+ ATPase, synthesis monovalent wheat germ lectin, isolation lymphocyte glycoproteins

Honors and Awards:
Two Invitational seminars: Department of Microbiology and Department of Biochemistry, Emory University, Atlanta, Georgia.

Publications: None

Serial No.: Z01-AG-00098-01-LCP
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Characterization of Alterations in Lymphocyte Membrane Structural Components

Previous Serial Number: None

Principal Investigator: J. W. Heine

Other Investigators: W. H. Adler

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	0.9
Others:	0.1

Project Description:

Objectives: To demonstrate that the observed reduction in cellular immune response with senescence is due to alterations in membrane structural components of the cells involved. The study primarily involves the membrane glycoproteins of spleen cells of various mouse strains and age groups stimulated with the mitogen succinyl-concanavalin-A (Succ-Con A).

Methods Employed:
1. Mitogenic stimulation and dose response to Succ-Con A assayed by microassay technique.

2. Immogenic demonstration of cell surface structural changes with senescence employed:
   a. Mixed lymphocyte culture (MLC) assay of different ratios of spleen cells from young and old mice of the same strain.
   b. MLC of spleen cells of young animals against mitomycin treated spleen cells from old animals and vice-versa.
   c. Cytotoxicity assay- ^{51}Cr. Spleen cells of young and old mice were labeled with ^{51}Cr and incubated with complement and antiserum against spleen cells of old mice prepared in both rabbits and in young mice of the same strain.

3. Acrylamide gel profiles of C^{14} glucosamine labeled plasma membrane preparations of partially purified spleen cells from young and old mice stimulated with Succ-Con A.

4. Quantitation of mitogenic response by viral infectivity center assay of Bloom, et al., (1973). Stimulated spleen cells are infected with VSV and assayed for infectivity centers on L-cell monolayers.

Major Findings:
1. The immunological approach to demonstrate cell surface alteration with senescence was minimally successful.

2. Preliminary acrylamide gel profiles of plasma membrane preparations indicate some difference in glycoprotein content between membrane preparations of young and old animals. However, the interpretation of the results is hindered by the minimal incorporation of C^{14} glucosamine by lymphocytes of old mice.

3. The difference in mitogenic response of a cell population as determined by the infective center assay is clearly due to a change in responding cells and not due to a change in metabolic activity. The finding that Succ-Con A stimulation induces the responding cells to produce an antiviral substance raises interesting questions: (i) What is its affect on the accuracy of the infective center assay? (ii) Is the quantity of antiviral substance produced by a stimulated cell population also dependent on the number of responding cells?

Significance to Biomedical Research and the Program of the Institute:
1. The above observations together with results of other researchers further substantiate that surface glycoprotein composition is altered with senescence. Furthermore, the augmentation of the stimulatory response by mercaptoethanol of lymphocytes enhances the feasibility of the study of membrane glycoproteins. The fact that mercaptoethanol can overcome the effect of senescence on the specific stimulatory response of lymphocytes (Makinodan, et al.) indicates that the otherwise nonresponding cells are metabolically normal except for the initial interaction between cell surface and mitogen, if one takes the theory on membrane-lectin interaction of Nicholson (1974) into consideration. Thus, the study on plasma membranes with senescence is of pertinence.

2. The finding that antiviral activity is produced by lymphocytes stimulated with Succ-Con A is significant with respect to resistance to viral infections with aging.

Proposed Course: In general, the research will follow as projected in the research project proposal. In addition, the antiviral substance produced by mitogenesis will be characterized with respect to known characteristics of interferon and its effect on the accuracy of the infectivity center assay as a mean to quantitate mitogenesis.

Honors and Awards: None

Publications: None

ANNUAL REPORT OF THE LABORATORY OF MOLECULAR AGING

Descriptive age-related changes have been accumulating in the literature for decades. Although these biologic phenomena may represent important initial steps in studying the biology of aging, the Laboratory of Molecular Aging has a continuing commitment to research that provide the basic scientific information needed to understand the mechanisms underlying these changes. In the past year, attention has focused on two critical areas known to undergo perturbations that lead to the inability of organisms to maintain homeostasis. These related to (A) Physiological Control Systems and (B) Genetic Information Transfer Systems.

(A) Physiological Control Systems

Our studies have impact on the mechanisms of age-dependent alterations in the following systems: (1) renal function; (2) muscle activity; (3) cardiac function; and (4) metabolism. The thrust of the work is directed towards problems and questions related to cell membranes, including: (a) molecular organization; (b) role in selective vectorial transport; (c) hormonal regulation of function; (d) catalytic function (e) turnover; and (f) failure to maintain structure, leading to cell death.

The kidney permits unique opportunities for studies on the molecular basis of the aging process. First, renal function itself is altered by age and, second, in other illnesses, renal adjustments to maintain fluid and solute homeostasis are slower in the aged. Techniques have been developed to isolate luminal (brush border) regions of the proximal tubule plasma membrane as osmotically active vesicles. The membranes are used as a model system to study mechanisms by which solutes are transported into the renal cell.

(a) Last year, a Na^+-dependent D-glucose uptake by the membranes was reported. Now, kinetic studies show that asymmetry of Na^+ across the membrane fully accounts for the up-hill transport of D-glucose by the proximal tubule. Imposition of an extra- to intra-vesicular Na^+-gradient causes a transient uptake of sugar to 10-fold the equilibrium value, indicating accumulation against a concentration gradient. The initial rate of uptake is enhanced 45x by 100 mM Na^+; other cations are not stimulatory. A single homogeneous Na^+-dependent glucose transport system is identified. Na^+ acts by decreasing the Km for glucose without altering Vmax. The action of Na^+ is dissected into a stimulatory effect when D-glucose and Na^+ are on the same side of the membrane (cis stimulation) and an inhibitory effect when sugar and Na^+ are on opposite sides of the membrane (trans inhibition).

(b) An important question of the Na^+-gradient model of glucose transport is whether Na^+-coupled glucose transfer is an electroneutral or electrogenic process. Two experimental approaches (1) effect of anions, and (2) use of specific ionophores, have been utilized to resolve this question. (1) With concentration gradients (medium > vesicle) for Na^+ salts of lipophilic anions the transient overshoot of D-glucose uptake above equilibrium is enhanced relative to that with NaCl. In contrast, Na^+ gradients with relatively impermeable anions results in no overshoot. With Na^+ salts of anions whose mode of membrane translocation are presumably electroneutral, i.e., acetate and

bicarbonate, uptake of D-glucose is not above equilibrium. These data indicate that it is the electrochemical gradient of Na^+ which is important and only anions capable of developing a membrane potential (interior negative) may support the Na^+-dependent D-glucose up-hill uptake. (2) The ionophore, gramicidin, which dissipates the Na^+ gradient, decreases D-glucose uptake. Nigericin, which effects an electroneutral exchange of Na^+ for H^+ or K^+, also decreases the Na^+-dependent D-glucose uptake. In contrast, valinomycin, which allows electrogenic K^+ conductance, stimulates D-glucose uptake, provided a (vesicle > medium) K^+ gradient is present. The proton conductor, FCCP, produces a stimulation of D-glucose uptake in the presence of a proton gradient (vesicle > medium). Valinomycin and FCCP alter the electrical potential across the vesicular membranes (make the interior negative). These results demonstrate that the Na^+-dependent transport of sugar by the renal tubule is an electrogenic process.

(c) The maintenance of acid-base balance by the kidney is critically important to the aged, especially when stressed. The three main acidifying processes, bicarbonate reabsorption, the generation of titratable acid and ammonia production are thought to be mediated by the common mechanism of H^+ secretion. The tubular mechanism underlying H^+ transport is unknown. Significantly, we find an ATPase, activated by HCO_3^- in luminal membranes. It is proposed that, in analogy with other membrane ATPases, hydrolysis of ATP by the renal membrane is coupled to the expulsion of protons into the tubular lumen. Protonation of HCO_3^- in the filtrate results in increased CO_2 which is then transported across the membrane. Hydration of CO_2 by cytosolic carbonic anhydrase leads to increased intracellular HCO_3^- which stimulates hydrolysis of ATP. Kinetic analysis of the enzyme indicates a Kactivation of 36 mM for HCO_3^-. Increases in concentrations of ATP or HCO_3^- lead to a higher Vmax without any change in Km, suggesting that the binding of ATP to the ATPase does not interfere with the subsequent binding of HCO_3^- and vice versa. HCO_3^- is relatively specific, although sulfite activates. Carbonic anhydrase increases HCO_3^--stimulated ATPase about 30% and this increase is blocked completely by the inhibitor Diamox.

(d) Maintenance of membrane potential and intra-/extra-cellular disequilibria of Na^+ and K^+ is essential for cell function. Na^+K^+ ATPase from eel electric organ membranes show: (1) Na^+ at low concentrations induce phosphorylation of the enzyme in the absence of K^+. The resulting phosphorylated product ($E \sim P$) is stable in acid; (2) low concentrations of K^+ convert some of the phosphorylated sites to a form that is labile in acid ($E \cdot P$), and high concentrations of K^+ convert approximately half of them to this form; (3) breakdown of the acid labile product is rate-limiting; (4) ratio of $[E \sim P]/[E \cdot P]$ remains at unity when ATP concentration is varied,. These findings suggest that the functional unit of the enzyme is a dimer, $E \sim P$ and $E \cdot P$ represent high affinity state for Na^+, and K^+, respectively, and the half units alternate out of phase with each other. Thus, while one unit picks up Na^+ and releases K^+, its neighbor picks up K^+ and discharges Na^+.

(e) Although the Na^+K^+ ATPase may function as the "Na^+-pump" effecting the decrease in intracellular Na^+, mechanisms must exist for the influx of Na^+. These processes are of importance not only for the maintenance of body salt-water balance but for the uptakes of sugars, amino acids, and bicarbonate, phosphate

and chloride anions which are coupled to the uptake of Na^+. Techniques have been developed for the study of Na^+ transport into renal tubular membranes and essential parameters for such studies, including temperature, incubation times, membrane and Na^+ concentrations, rapid quench procedures and different anions, have been defined.

(f) Last year, the Na^+-dependent uptake of the neutral amino acid, alanine, by renal membranes was reported. Kinetic studies now demonstrate that alanine transport can be dissected into two processes: (1) a stereospecific, saturable, system dependent on the electrochemical Na^+ gradient; and (2) a non-saturable system, which does not distinguish between L- and D-alanine, and is Na^+-independent. Up-hill transport of alanine is mediated via the Na^+-dependent system. Increases in the electrochemical gradient of Na^+ enhance uptake. Of significance, the uptakes of L-alanine and D-glucose by the luminal membrane mutually compete for the electrochemical Na^+-gradient. This explains the observation that the transports of sugars and amino acids in the intact kidney are mutually inhibitory.

(g) Genetic defect analyses in man as well as physiological studies with various intact preparations have led to the concept for at least four major amino acid transport systems in kidney (and other tissues): the neutral, basic, acidic, and imino-glycine systems. These studies have been unable to distinguish events occurring at the luminal membrane from those taking place at the basal-lateral membrane. We have taken advantage of our preparation to examine the transport of the basic amino acid arginine across the luminal membrane. We find: (1) the presence of a positively charged guanido group is a prerequisite for the recognition of arginine by the apical membrane; (2) external Na^+ plays no role in arginine uptake; (3) counter movement of a positive charge in the form of H^+, or K^+ or Na^+, stimulates influx of arginine; (4) the membrane is stereospecific for L-arginine; and (5) arginine uptake is inhibited by lysine and ornithine but not by neutral and acidic amino acids, and imino acids. These data are consistent with the hypothesis that the basic aminoaciduria disorders observed in man may result from defects at the luminal membrane of the proximal tubule.

The ability of tissues, e.g., skeletal muscle, heart, to perform maximally declines with age. A decreased capacity to meet the demands of bioenergetic processes may be one factor responsible for this deterioration with senescence. We previously reported that mitochondria in muscle (blowfly flight muscle) in situ show age-related membrane damage and these ultrastructural alterations are correlated with biochemical decrements in the maximally stimulated (ADP-induced) respiration. We now find that mitochondria from aged blowflies show similar decreases in rates of uncoupled (FCCP-stimulated) respiration. This suggests that the age-dependent defect is within the oxidative pathway, and not with phosphorylation. Partial reactions of electron transport and contents of cytochromes further identify the site of the age-dependent defect. Specific activity of the "latent" ATPase is increased in mitochondria from senescent blowflies. Perhaps of significance are lipid analyses of flight muscle mitochondria showing that mitochondria with the greatest decline with age are those deficient in Vitamin E, a natural component of membranes presumably having a role as an antioxidant.

Genetic probes have been used in studying age changes in insect flight muscle. "Null" mutations at NAD-linked α-glycerolphosphate (αGP) dehydrogenase locus were isolated from Drosophila after mutagenesis. Mutants have essentially no dehydrogenase activity and severely restricted flight ability. After 25 generations, one stock regained the ability to fly despite the continued absence of measurable enzyme activity. Heterozygotes of three noncomplementing αGpdh "null" alleles and the "adapted" homozygote were examined for enzymatic activities related to flight metabolism, ultrastructure of flight muscle and longevity. Mitochondrial oxidations in the mutant genotypes in early adult life were indistinguishable from those of the wild type. Two weeks after eclosion, however, there is a premature deterioration and atrophy in ultrastructure and biochemical properties of mitochondria in the "null" mutants, seen previously only in aged flies. The morphological and biochemical changes are accompanied by the premature mortality of the mutant heterozygotes. "Adapted" flies are essentially identical with wild type in each of the aging characters. One possible explanation for these genetically determined aging changes may be a disuse hypothesis. Mutants being unable to synthesize αGP have decreased function for mitochondrial αGP oxidation. If the rapid turnover of flavoprotein and cytochromes in response to substrate is necessary for structural integrity of mitochondria, then αGpdh mutations would result in age-dependent mitochondrial atrophy.

An essential step in understanding the mechanisms by which heart muscle contractibility changes with age is to define the transport processes which are basic to regulation of cardiac function. One system being investigated is the transport and intracellular compartmentation of Ca^{2+} by the sarcoplasmic reticulum (SR). An initial comparison of uptake by 6 and 24 month old rats shows that Vmax remains constant but that Km decreases in the older population. This apparent decrease in affinity for Ca^{2+} is consistent with an increased contraction duration observed in isolated aged myocardium.

In addition to the SR, the heart has a well-developed Ca^{2+} pumping system in mitochondria which serves to regulate intracellular Ca^{2+}. The interaction of heart mitochondria with Ca^{2+} may have important implications in other areas related to cardiac physiology. We previously reported on the crucial role of Ca^{2+} in activating phosphorylase b kinase, thus coupling glycogenolysis to myofibrillar contraction. Also, that cardiac mitochondrial pyruvate dehydrogenase, which regulates the oxidations of carbohydrate and lactate via the tricarboxylate cycle, exists in phosphorylated (inactive) and dephosphorylated (active) forms which are interconverted by a kinase and phosphatase. We now find that Mg^{2+} and Ca^{2+} are potent activators of pyruvate dehydrogenase phosphatase, thus reactivating the inactive enzyme. Maximal activation is obtained with 0.5 mM Mg^{2+} and 10^{-6} to 10^{-7} free Ca^{2+}.

Investigations of hormonal regulation of physiological control systems have focused on the interactions of cyclic nucleotides, cAMP and cGMP, with membrane target sites. Using renal membranes as a model, we have examined (1) synthesis of cAMP by adenyl cyclase; (2) binding of cAMP and cGMP to membrane receptors; (3) cAMP-dependent and -independent protein kinases in the membrane; (4) nature of endogenous membrane proteins that are phosphorylated by kinases; (5) actions of cAMP and cGMP phosphodiesterases that regulate levels of cyclic nucleotides; (6) role of cytosolic protein factors and divalent cations in both inhibiting

the catalytic function of cyclic AMP-dependent protein kinases and in activating the hydrolysis of cAMP and cGMP; and (7) how phosphorylated-dephosphorylated membrane regulates transport, the physiological action of hormones, e.g., catecholamines, vasopressin, parathyroid hormone and calcitonin.

Studies to delineate the regulatory mechanism in metabolism are essential to understanding changes that occur in altered physiological states, including aging, obesity, atherosclerotic disease, etc. The work this year has centered on the control of the tricarboxylate (TCA) cycle in skeletal muscle and cardiac mitochondria. The general approach has been to determine steady-state concentrations of key intermediates of the cycle, and ATP/ADP and NADH/NAD ratios, and the response of these concentrations and ratios to changes in flux in the pathway induced either in isolated mitochondria or in intact tissue. The TCA cycle is the focal point where carbohydrate, amino acids and fatty acids meet and it represents the site where metabolism is integrated and intensely regulated. Oxidation of pyruvate (from carbohydrates), amino acids, and fatty acids have been examined separately and when in combination. As an example, on activating respiration in cardiac mitochondria oxidizing lipids, palmitoylcarnitine or octanoate, steady state metabolite concentrations implicate a limitation in the oxidation of lipid at the level of the TCA cycle rather than of β-oxidation. Regulation of the cycle is most pronounced at citrate synthase, which is controlled by the availability of oxaloacetate, and this is affected mainly by the ratio NADH/NAD. The intramitochondrial concentration of citrate is important, as citrate competes with oxaloacetate. Citrate, in equilibrium with isocitrate, is controlled by isocitrate dehydrogenase. In heart, this enzyme is controlled by NADH/NAD, and not ATP/ADP as found in other tissues.

(B) Genetic Information Transfer Systems

Our research program focuses on the interaction of molecules that are concerned in the propagation of genetic information. The interactions of DNA and RNA with each other, with proteins, and with metal ions are studied with the object of understanding these interactions in terms of biological function, particularly in replication of DNA, transcription of RNA, and protein synthesis. A primary objective is to determine under what conditions metal ions are essential for information transfer, and under what conditions they produce errors in information. It is sought to determine the significance of metal ions in biological aging. Synthetic analogs of nucleic acids are used to probe the nature of viral infection, its changes with cellular aging, and to determine how the uptake of foreign macromolecules by cells is affected by cellular aging; it is hoped in this way to produce pharmaceutical agents to offset deleterious age changes. Studies are carried out to elucidate the biological role of important substances like hemoglobin and how structural changes in these substances can lead to aging impairment.

Cellular protein synthesis occurs on ribosomal particles, on which the genetic message from mRNA is translated through recognition of tRNA molecules that are designed for carrying specific amino acids. To understand protein synthesis, and how it may be altered, one must understand the interaction between ribosome, mRNA, and tRNA. We have developed a circular dichroism technique for measuring subtle changes in ribosomal conformation, and find that mRNA modifies ribosomal structure by binding to it at sites in addition to the recognition site between

the RNA molecules. Binding of messenger to ribosomes paves the way for subsequent binding to tRNA, which produces a change in the ribosome only if message is added first. If the tRNA is added first, it does not affect the ribosome and it prevents the mRNA from achieving its proper modification. Thus each event on the ribosome achieves a desired conformational change when and only if it appears in the intended sequence.

We previously demonstrated that divalent metal ions in concentrations not much higher than those required for biological processes induce mispairing of nucleotide bases. Mispairing can produce errors in replication, transcription, and translation. We now show that the mispairing phenomenon can be reversed by raising the temperature. Thus errors produced by metal ions can be countered. Ribosomes from different species have different degrees of fidelity in translation, when challenged by metal ions. Such differences can result from differences in ability to counteract the effects of metal ions.

Turning the genome on and off is partly controlled by proteins bound to DNA. If this control mechanism is to operate properly the proteins must fit the DNA in the desired manner. We find that many polypeptides react with DNA to form two different structures when the components of the reaction are brought together by either (1) directly mixing (DM), or (2) prior mixing at a high ionic strength which keeps them from complexing, followed by gradient dialysis (GD) to bring the ionic strength to complexing conditions. GD and DM produce different structures for a variety of DNA-polypeptide complexes, but only one structure for the DNA complex with histone I. Failure of histone I to assume a metastable structuure indicates that a time lag exists between formation of various DNA-histone bonds, thus permitting the complex spontaneously to assume the thermodynamically favored conformation. Histone IV resembles synthetic polypeptides in that it forms a metastable complex with DNA. Conformational changes in histone IV binding are thus more likely than changes in histone I binding to lead to errors in transcription.

Holliday et al. described an increase in concentration of altered proteins in aged cells. If this is due to errors in protein synthesis, challenge of cells with virus should result in a corresponding increase in altered viral proteins. Such a challenge of WI38 cells was carried out with polio virus. No proportionate change in viral proteins was observed. It is concluded that the Holliday alterations are not due to errors in protein synthesis. Post-transcriptional changes in proteins can better explain the results.

Polyvinyladenine inhibits reverse transcriptase, an enzyme involved in replication of leukemia virus. A mechanistic study of this inhibition indicates that base pairing ability may not be involved. This finding enlarges the class of polymers with possible antiviral activity. To prove this hypothesis several polymers were synthetized; one, poly(9-vinylpurine), is a very potent inhibitor and it also blocks replication of leukemia virus.

Changes in the amount of oxygen bound to hemoglobin change the conformation of the protein. We investigated the oxidation induced by Cu(II) as a function of oxygen pressure. The major effects of the conformation change can be attributed to an increased affinity of copper at the hemoglobin site required for oxidation, when the oxygen is released. Thus, hemoglobin is more susceptible to Cu(II) oxidation in venous than in arterial blood.

Serial No. Z01 AG 00041-02 LMA
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Section on Intermediary Metabolism
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mechanisms of the Age-Dependent Alterations in Physiological Control Systems. I. Biology of Renal Plasma Membranes.

Previous Serial Number: HD-GMA-13

Principal Investigator: Bertram Sacktor, Ph.D.

Other Investigators: Jeanne Beck, Ph.D.; Arthur Chernoff, M.D.; Steven Fass, M.D.; Marc Hammerman, M.D.; Tony Liang, Ph.D.

Man Years:

Total:	7.3
Professional:	5.3
Other:	2.0

Project Description:

Objectives: These studies are targeted to provide the basic scientific information needed to understand the mechanisms whereby age-dependent perturbations in physiological control systems lead to the inability of the organism to maintain homeostasis. The research has impact on the mechanism underlying age-related changes in renal function. The thrust of the work is focused on questions dealing with the biology of membranes, including: (1) molecular organization; (2) catalytic properties; (3) selective vectorial transport; (4) hormonal regulation of function; (5) turnover; and (6) failure to maintain structure and function, leading to cell death.

Methods Employed: The apical (luminal) segment of the renal proximal tubule plasma membrane, the renal brush border, is used as a model membrane to study mechanisms of transport.

Major Findings: Renal brush borders were isolated as osmotically active vesicles (3) and the membranes were used as a model system to study mechanisms by which solutes are transported selectively and vectorially by the proximal tubular cell (5). A single homogeneous stereospecific Na^+-dependent D-glucose transport system was identified (1,4). Imposition of an extra- to intravesicular Na^+-gradient resulted in the transient uptake of the sugar to 10-fold the equilibrium value, demonstrating accumulation against a concentration gradient (2). Na^+ acts by decreasing the K_m for D-glucose without altering V_{max}. The action of Na^+ was dissected into a stimulatory effect when D-glucose and Na^+ were on the same side of the membrane (cis stimulation) and an inhibitory effect when sugar and Na^+ were on opposite sides of the membrane (trans

inhibition). The energetics of the Na^+-dependent transport of D-glucose was studied by determining how alterations in the electrochemical potential of the renal membrane induced by anions, ionophores and a proton conductor affect the uptake of the sugar (3). The results indicate that transport of D-glucose into membrane vesicles is an electrogenic process. It is suggested that in the intact kidney the asymmetric distribution of Na^+ across the proximal tubular cell and the electrochemical potential across the luminal membrane provide the driving force to transport D-glucose against its concentration gradient.

The maintenance of acid-base balance by the kidney is critically important to the aged, especially when stressed. The three main acidifying processes, bicarbonate reabsorption, the generation of titratable acid and ammonia production are thought to be mediated by the common mechanism of H^+ secretion. The tubular mechanism underlying H^+ transport is unknown. Significantly, we found an ATPase, activated by HCO_3^- in luminal membranes. It is proposed that, in analogy with other membrane ATPases, hydrolysis of ATP by the renal membrane is coupled to the expulsion of protons into the tubular lumen. Protonation of HCO_3^- in the filtrate results in increased CO_2 which is then transported across the membrane. Hydration of CO_2 by cytosolic carbonic anhydrase leads to increased intracellular HCO_3^- which stimulates hydrolysis of ATP. Kinetic analysis of the enzyme indicated a $K_{activation}$ of 36 mM for HCO_3^-. Increases in concentrations of ATP or HCO_3^- led to a higher V_{max} without any change in K_m, suggesting that the binding of ATP to the ATPase does not interfere with the subsequent binding of HCO_3^- and *vice versa*. HCO_3^- was relatively specific, although sulfite activated. Carbonic anhydrase increased HCO_3^--stimulated ATPase about 30% and this increase was blocked completely by the inhibitor Diamox.

Kinetic studies (Fass, Fed. Proc. Abstr. 1975) on the Na^+-dependent uptake of the neutral amino acid, alanine, by renal brush border membranes demonstrate that alanine transport can be dissected into two processes: (1) a stereospecific, saturable, system dependent on the electrochemical Na^+ gradient; and (2) a non-saturable system, which did not distinguish between L- and D-alanine, and was Na^+-independent. Uphill transport of alanine was mediated via the Na^+-dependent system. Increases in the electrochemical gradient of Na^+ enhanced uptake. Of significance, the uptakes of L-alanine and D-glucose by the luminal membrane mutually competed for the electrochemical Na^+-gradient. This explains the observation that the transports of sugars and amino acids in the intact kidney are mutually inhibitory.

Genetic defect analyses in man as well as physiological studies with various intact preparations have led to the concept for at least four major amino acid transport systems in kidney (and other tissues): the neutral, basic, acidic, and imino-glycine systems. These studies have been unable to distinguish events occurring at the luminal membrane from those taking place at the basal-lateral membrane. We have taken advantage of our preparation to examine the transport of the basic amino acid arginine across the luminal membrane (Hammerman, Fed. Proc. Abstr. 1975). We found: (1) the presence of a positively charged guanido group was a prerequisite for the recognition of arginine by the apical membrane; (2) external Na^+ played no role in arginine uptake; (3) counter movement of a positive charge in the form of H^+, or K^+ or Na^+, stimulated influx of arginine; (4) the membrane was stereospecific for L-arginine; and (5) arginine uptake was inhibited by lysine and ornithine but not by neutral and acidic amino acids, and imino acids. These data are consistent with the

hypothesis that the basic aminoaciduria disorders observed in man may result from defects at the luminal membrane of the proximal tubule.

Studies on the mechanism for the influx of Na^+ across renal membrane vesicles was initiated because this process is of importance not only for the maintenance of body salt-water balance but for the uptakes of sugars, amino acids, and bicarbonate, phosphate and chloride anions which are coupled to the uptake of Na^+. Techniques were developed for the study of Na^+ transport and essential parameters including temperature, incubation times, membrane and Na^+ concentrations, rapid quench procedures and different anions, were defined.

Significance to Bio-medical Research and the Program of the Institute: These studies provide the basic scientific information needed to understand the mechanisms whereby age-dependent perturbation in physiological control systems, using renal function as a model, lead to the inability of the aged organism to maintain homeostasis.

Proposed Course: Age associated alterations in the biology of membranes will be continued by studying mechanisms for the development of transmembrane electrochemical potentials which provide the driving force for uphill solute translocation; the nature of the coupling between Na^+ flux and the movements of other solutes; and mechanisms of H^+ secretion and HCO_3^- and $H_2PO_4^-$ reabsorption.

Keyword Descriptors:

Membrane transport, renal brush border, plasma membranes, sugar transport, amino acid transport, ATPases, biology of aging.

Honors and Awards:

Dr. Sacktor presented invitational seminars to various academic and research institute groups.

Publications:

1. Aronson, P. and Sacktor, B.: Transport of D-Glucose by Brush Border Membranes Isolated from the Renal Cortex. Biochim. Biophys. Acta 356: 231-243, 1974.

2. Aronson, P. and Sacktor, B.: The Na^+ Gradient Dependent Transport of D-Glucose in Renal Brush Border Membranes. J. Biol. Chem., in press.

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4. Mitchell, M., Aronson, P. and Sacktor, B.: Further Studies on the Previously Proposed Saturable High Affinity Site for D-Glucose in Renal Brush Border Membrane Preparations. J. Biol. Chem. 249: 6971-6975, 1974.

5. Sacktor, B., Chesney, R., Mitchell, M. and Aronson, P.: The Interactions of D-Glucose with the Renal Brush Border. In Fanelli, G. and Wesson, L. (Eds.): Recent Advances in Renal Physiology and Pharmacology. Baltimore, University Park Press, 1974, pp. 13-26.

Serial No. Z01 AG 00042-02 LMA
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Section on Intermediary Metabolism
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mechanisms of the Age-Dependent Alterations in Physiological Control Systems. II. Hormonal Regulation of Membrane Function.

Previous Serial Number: HD-GMA-13

Principal Investigator: Bertram Sacktor, Ph.D.

Other Investigators: Charles Filburn, Ph.D.; Edward George, M.D.

Man Years:

Total:	5.3
Professional:	2.3
Other:	3.0

Project Description:

Objectives: These studies are targeted to provide the basic scientific information needed to understand the mechanisms whereby age-dependent perturbations in physiological control systems lead to the inability of the organism to maintain homeostasis. The research has impact on the mechanism underlying age-related changes in renal and cardiac function. Investigations are focused on the biochemical interactions of hormones, mediated via cyclic AMP and cyclic GMP, with membrane systems.

Major Findings: We have examined (1) synthesis of cAMP by adenyl cyclase; (2) binding of cAMP and cGMP to membrane receptors; (3) cAMP-dependent and -independent protein kinases in the membrane; (4) nature of endogenous membrane proteins that are phosphorylated by kinases; (5) actions of cAMP and cGMP phosphodiesterases that regulate levels of cyclic nucleotides; (6) role of cytosolic protein factors and divalent cations in both inhibiting the catalytic function of cyclic AMP-dependent protein kinases and in activating the hydrolysis of cAMP and cGMP; and (7) how phosphorylated-dephosphorylated membrane regulates transport, the physiological action of hormones, e.g., catecholamines, vasopressin, parathyroid hormone and calcitonin.

Isolated renal proximal tubule plasma membranes possess a highly active, hormone sensitive, adenyl cyclase, generating sufficient cAMP to account for the subsequent action of the cyclic nucleotide.

The kinetics of binding of cAMP to isolated renal brush border membrane was studied in detail since this process incorporates the interaction of cAMP with a regulatory subunit of a membrane-bound protein kinase as well as the mechanism by which cAMP, generated predominantly on the antiluminal membrane,

traverses the luminal membrane and appears in the urine. The rates of binding and debinding were temperature sensitive; the final levels were identical. The cAMP bound to the membrane was recovered unchanged. These findings, plus the observation that, when bound, cAMP was resistant to hydrolysis by endogenous membrane or exogenously added phosphodiesterase, suggests that one of the components of the mechanism for reversing the response of the membrane to cAMP is the dissociation of the nucleotide from the membrane. Binding was only partially saturable with respect to cAMP concentration, apparently with more than one binding site. These results are consistent, however, with the association of cAMP with a regulatory subunit of protein kinase. The binding to the membrane was relatively specific for cAMP. Renal membrane did bind cGMP, but this binding was relatively non-specific.

Protein kinase and phosphoprotein phosphatase activities were demonstrated in renal brush border membranes. Phosphorylated serine and threonine residues on the membrane protein were identified. The autophosphorylation of the membrane protein was cAMP-independent and not inhibited by a specific protein inhibitor of cAMP-dependent protein kinases. Membrane phosphorylation of histones (f2b and mixed) was cAMP-dependent. cAMP maximally stimulated at 10^{-6} to 10^{-5} M with K_a for cAMP being 7 x 10^{-8} M. Double reciprocal plots of rate vs ATP concentration were non-linear and cAMP caused the lowering of the apparent K_m for ATP, a result compatible with multiple enzymes with differing substrate (ATP) affinity and cAMP responsiveness.

Concentrations of cAMP and cGMP in tissues are controlled by the activities of phosphodiesterases. Two activities in renal plasma membranes were distinguished based on properties observed upon assaying at 10^{-3} or 10^{-6} M cAMP (Filburn, Fed. Proc. Abstr. 1975). At 10^{-3} M, the phosphodiesterase was optimally active at pH 8; activated by 10 mM divalent metals in the order Co> Mg = Mn > Ca; heat stable; inhibited markedly by EGTA and EDTA, and reactivated by Ca > Mg; inhibited by theophylline, cGMP and cIMP; showed 4-fold greater activity toward cAMP than cGMP; and was markedly inhibited by a heat stable factor that is sensitive to neuraminidase and phospholipase but not to proteases. In contrast, at 10^{-6} M cAMP the enzyme had a pH optimum of 6-7; activated by Co, Mg and Mn, but inhibited by Ca; heat labile; inhibited by EDTA but slightly by EGTA, and reactivated by Mg but not Ca. Phosphodiesterase activities in the cytosol were distinguished from the enzymes in the membrane. The soluble enzymes are strongly activated by a heat stable protein and markedly inhibited by Ca.

Significance to Biomedical Research and the Program of the Institute: These studies provide the basic scientific information needed to understand the mechanisms by which age-dependent perturbations in the hormonal regulation of physiological systems lead to the inability of the aged organism to maintain homeostasis.

Proposed Course: The mechanisms by which age affects the organisms response to hormones, with particular relation to membrane function and the roles of cAMP and cGMP, will be continued by studying the interactions of cyclic nucleotides with protein kinases and phosphoprotein phosphatases, the enzymes which respond to changes in the concentrations of "second messengers." The mechanisms whereby tissue levels of cyclic nucleotides are controlled by phosphodiesterases, whose activities, in turn, are modulated by endogenous factors

and divalent cations, will be investigated. It is proposed to extend the studies of these processes in kidney to the heart of aged animals, where synchronous, but reciprocal, changes in the levels of cAMP and cGMP with heart beat have been suggested.

Keyword Descriptors:

cAMP, cGMP, phosphodiesterase, renal membranes, protein kinase, phosphoprotein phosphatase, hormones, biology of aging.

Honors and Awards: None

Publications:

Insel, P., Balakir, R. and Sacktor, B.: The Binding of Cyclic AMP to Renal Brush Border Membranes. J. Cyclic Nucleotide Res. 1: 107-122, 1975.

Serial No. Z01 AG 00043-02 LMA
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Section on Intermediary Metabolism
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mechanisms of the Age-Dependent Alterations in Physiological Control Systems. III. Biology of Mitochondrial Membranes and the Regulation of Skeletal Muscle Activity, Cardiac Function and Metabolism.

Previous Serial Number: HD-GMA-13

Principal Investigator: Bertram Sacktor, Ph.D.

Other Investigators: Bernard Bulos, Ph.D.; Richard Hansford, Ph.D.; Roger Johnson, Ph.D.

Man Years:

Total:	6.4
Professional:	3.4
Other:	3.0

Project Description:

Objectives: These studies are targeted to provide the basic scientific information needed to understand the mechanisms whereby age-dependent perturbations in physiological control systems lead to the inability of the organism to maintain homeostasis. The research has impact on the mechanism underlying age-related changes in skeletal muscle activity, cardiac function and metabolism. The thrust of the work is focused on questions dealing with the biology of membranes, including: (1) molecular organization; (2) catalytic properties; (3) selective vectorial transport; (4) hormonal regulation of function; (5) turnover; and (6) failure to maintain structure and function, leading to cell death.

Methods Employed: As model systems, mitochondria isolated from insect flight muscle and mammalian cardiac muscle are employed.

Major Findings: Mitochondria isolated from senescent blowflies showed age related membrane damage and these ultrastructural alterations were correlated with biochemical decrements (1,14).

Genetic probes were used to study aging change in insect flight muscle (10). Drosophila with "null" mutations showed premature deterioration and atrophy in mitochondrial membrane structure and biochemical properties, which were

accompanied by premature mortality. "Adapted" flies were essentially identical with wild type in each of the aging characters.

The mechanisms by which the tricarboxylate cycle is controlled in insect flight muscles and cardiac muscles were described (2-7,9,10-13). The crucial role of Ca in the coupling of bioenergetic and contractile activities was demonstrated.

Mitochondrial-cytosol interactions in the control of glucose uitlization in cerebral tissue was shown (8).

Significance to Biomedical Research and the Program of the Institute: These studies provide the basic scientific information needed to understand the mechanisms by which the ability of tissues, e.g. skeletal muscle, heart, to perform maximally declines with age.

Proposed Course: The effect of age on the metabolic control in skeletal muscle will be studied using insect flight muscle as a model system. It is planned to investigate the mechanism of Ca translocation in mitochondria from mature and senescent animals to correlate with age-dependent alteration in cardiac function.

Keyword Descriptors:

Bioenergetics, intermediary metabolism, mitochondria, tricarboxylate cycle, biology of aging.

Honors and Awards:

Dr. Sacktor presented invitational seminars to various academic and research institute groups.

Publications:

1. Bulos, B., Shukla, S. and Sacktor, B.: Bioenergetics of Mitochondria from Flight Muscles of Aging Blowflies: Partial Reactions of Oxidation and Phosphorylation. Arch. Biochem. Biophys. 166: 639-644, 1975.

2. Chiang, P. and Sacktor, B.: Control of Pyruvate Dehydrogenase Activity in Intact Cardiac Mitochondria. Regulation of the Inactivation and Activation of the Dehydrogenase. J. Biol. Chem. 250: 3399-3408, 1975.

3. Hansford, R.: The Control of Tricarboxylate Cycle Oxidations in Blowfly Flight Muscle. The Steady State Concentrations of Coenzyme A, Acetyl Coenzyme A and Succinyl Coenzyme A in Flight Muscle and Isolated Mitochondria. Biochem. J. 142: 509-519, 1974.

4. Hansford, R.: The Control of Tricarboxylate Cycle Oxidations in Blowfly Flight Muscle. The NAD^+ and NADH Content of Flight Muscle and Isolated Mitochondria, the ATP and ADP Content of Mitochondria, and the Energy Status of the Mitochondria During Controlled Respiration. Biochem. J. 146: 537-547, 1975.

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8. Hochman, M., Shimada, Y. and Sacktor, B.: Solubilization of Brain Mitochondrial Hexokinase by ATP and Glucose-6-phosphate: Ultrastructural Aspects. J. Neurochem. 23: 861-863, 1974.

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12. Sacktor, B.: Biochemistry of Insect Flight. Part I: Utilization of Fuels by Muscle. In Candy, D. J. and Kilby, B. A. (Eds.): Insect Biochemistry. London, Chapman and Hall Ltd., in press.

13. Sacktor, B.: Biochemical Adaptations for Flight in the Insect. Biochem. J. (Transactions), in press.

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Serial No. Z01 AG 00044-02 LMA
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Effects of Metal Ions on the Structure and Function of Nucleic Acids

Previous Serial Number: HD-GMA-12

Principal Investigator: G. Eichhorn, P. Clark, J. Pitha, J. Rifkind

Other Investigators: None

Man Years:

Total:	3.3
Professional:	2.0
Other:	1.3

Project Description:

Objectives: (1) To understand the participation of metal ions in the biological activities of nucleic acids, (2) to obtain clues to the reasons for the toxicity of metal ions, (3) to understand the role of metal ions in the aging process, (4) to use metal ions as probes to study the mechanism of biological information transfer.

Methods Employed: (1) Effects of metal ions are studied to determine under what conditions they serve as essential function in information transfer, and under what conditions they induce errors in the information content. (2) Metal ion effects are studied in tissue culture to determine intracellular biological activity.

Major Findings: A. Reversibility of mispairing of nucleotide bases induced by metal ions. We have previously shown that metal ions, e.g. Mg^{2+}, which are required in the normal processes involving nucleic acids, will induce mispairing of nucleotide bases when present in slight excess of the required quantity. Such mispairing could lead to errors in replication, transcription, or translation. Mispairing had been demonstrated by the use of three systems: Poly(A)·poly(I,U), poly(I)·poly(A,C), and poly(I)·poly(U,C). All of these systems have been reinvestigated to determine whether it is possible to reverse mispairing, once induced by metal ions. Using nuclear magnetic resonance line sharpening techniques, it was shown that mispairing is reversible in all three systems by raising the temperature. This is consistent with the mechanism for metal induced mispairing, namely, that it is caused by the increased stability

of polynucleotide interactions, thus precluding sensitivity toward the most stable (complementary) base pairs. By introducing another parameter that will destabilize polynucleotide interaction, such as increased temperature, sensitivity toward the complementary base pairs can then be restored. The reversibilities of metal induced mispairing of bases is of significance because it indicates that the error-producing effects of the metal ions can be countered by other parameters. Ribosomes from different species have different degrees of fidelity in translation when challenged by metal ions. Our experiments have shown that such differences can result from differences in the ability to counteract the effects of the metal ions.

B. Metal ions in young and old cells. There is no consistency in the techniques and reporting of the metal content of biological tissue. We are attempting to utilize techniques and reporting that are reproducible and meaningful. Results have been obtained under conditions of maximum washing on frozen WI-38 cell packs from Dr. Leonard Hayflick. Under these conditions some metal ions could be removed from the cell; what is reported are therefore concentrations of strongly bound metal. The results, calculated on the basis of metal content/DNA, which is a measure of metal content/cell, indicate that the following metal ions accumulate with age: K^{+}, Mg^{2+}, Ca^{2+}, Fe^{2+}, Zn^{2+}. On a weight basis there is little change in metal content with age.

C. Reaction of platinum complexes with DNA. The dramatic biological activities of the platinum complexes have been of interest to us because of the usefulness of the complexes as probes of biological mechanisms. We have previously shown that Pt bound to DNA can act as a stop sign in RNA synthesis, leading to small message units. Recent clues to the nature of the binding to DNA have come from degradation studies on DNA-Pt complexes and analysis of degradation products. The results indicate that with cis $[Pt(NH_3)_2Cl_2]$ all of the Pt is bound to guanosine and none to any of the other nucleosides. With trans $[Pt(NH_3)_2Cl_2]$ binding is also mostly to guanosine, with slight binding to cytosine, indicating that the trans complex is engaged in crosslinking of bases in the GC region of DNA. These results are in line with the interpretation of earlier results that cis complexes stop transcription by chelation, and trans complexes act through a crosslinking mechanism.

D. Competition between metal binding and ordered helical structure of polynucleotides. That Cu(II) also forms crosslinks between polynucleotide chains has now been unequivocally demonstrated by the finding that the Cu(II) activity necessary to produce cooperative disordering depends upon the polymer concentration. Cu(II) binding has also been used to detect differences between polynucleotides in their tendency to form helices. The helix → coil transition, for example, occurs at lower Cu(II) concentration for poly(C) than for poly(A). With poly(C) the transition is much more cooperative at low polymer concentration than for poly(A). These differences are explained by a relatively higher affinity of Cu(II) for poly(C), a lesser tendency of poly(A) to surrender helical structure when challenged by Cu(II), and a greater tendency for poly(A) to produce small intramolecular loops. We believe that these differences are of significance for the interpretation of the presence of poly(A) units in the regulation of genetic message.

Significance to Bio-medical Research and to the Program of the Institute:
The participation of metal ions in every aspect of genetic information transfer makes the study of the interaction of metal ions with the nucleic acids of major importance in understanding the perturbation of the genetic information that is presumably characteristic of the aging process. It is likely that metal ions can have a direct effect on biological aging.

Proposed Course of Project. It is intended to continue studies to determine the sites of binding of metal ions on nucleic acids, the conformational changes induced by such binding, and to correlate these effects with biological functions. We shall continue to evaluate age changes in the metal content of WI-38 cells, with the eventual goal of establishing the impact of these changes on cellular biochemistry. We shall study errors induced by metal ions in replication and transcription.

Keyword Descriptors: Toxicity, aging, mispairing, nucleotide bases, nuclear magnetic resonance, polynucleotide, fidelity, crosslinking

Honors and Awards:
G. Eichhorn was appointed to the Editorial Board of the Journal of Molecular Catalysis.

G. Eichhorn was invited to participate in the Dahlem Workshop on the Nature of Seawater, Berlin, Germany, March, 1975.

G. Eichhorn was invited to present seminars at many universities.

Publications:
Clark, P. and Eichhorn, G. L.: A Predictable Modification of Enzyme Specificity. Selective Alteration of DNA Bases by Metal Ions to Promote Cleavage Specificity by Deoxyribonuclease. Biochemistry 13:5098-5102 (1974).

Butzow, J. J. and Eichhorn, G. L.: Different Susceptibility of DNA and RNA to Cleavage by Metal Ions. Nature 254:358-359 (1975).

Eichhorn, G. L.: Active Sites of Biological Macromolecules and their Interaction with Heavy Metals. In "Ecotoxicology of Heavy Metals and Organic Halogen Compounds", J. Mills, ed., Plenum Press, in press (1975).

Eichhorn, G. L.: Organic Ligands in Natural Systems, in "Dahlem Workshop on the Nature of Seawater, ed. by E. D. Goldberg, Pergamon Press, Oxford, in press (1975).

Serial No. Z01 AG 00045-02 LMA
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Structures of Nucleoproteins and Ribosomes, and Interaction of Protein Synthesis Components

Previous Serial Number: HD-GMA-3

Principal Investigator: G. Eichhorn, J. Butzow, Y. Shin

Other Investigators: None

Man Years:

Total:	3.0
Professional:	2.3
Other:	0.7

Project Description:

Objectives: (1) To determine specificities in the binding of proteins to nucleic acids that are responsible for biological activity, (2) to elucidate the structure of chromatin, (3) to understand how aging may be related to nucleoprotein structure, (4) to study the structure of the ribosome and its interaction with other substances engaged in protein synthesis.

Methods Employed: (1) Chromatin structure is analyzed by means of model systems to determine which characteristics of chromatin are responsible for its function. (2) The interaction of ribosomes, messenger RNA, transfer RNA and other factors involved in protein synthesis are studied by optical rotatory dispersion, circular dichroism, and tracer analysis.

Major Findings: A. Conformational changes in the interaction of ribosome, messenger RNA, and transfer RNA. The mechanism of protein synthesis as well as age alterations in this mechanism are keyed to subtle changes in the structure of the ribosomal particles on which protein synthesis occurs. We have refined a circular dichroism (CD) technique to measure these subtle changes. The successive binding of poly(U)—which codes for phenylalanine—and then phenylalanine-specific transfer RNA ($tRNA^{Phe}$) to E.coli ribosomes results in a two-step conformational change in the ribosome. These changes require poly(U) of long chainlength; a U-octamer produces no significant CD change. It is concluded that the ribosomal changes involve not only the codon-anticodon interaction site, but other points of interaction between messenger and ribosome. In the absence of poly(U), t-RNA

produces no significant CD effect on the ribosome; moreover, initial exposure of the ribosome to t-RNA inhibits the change induced by poly(U), which is reduced in magnitude. These results suggest that binding of messenger RNA to ribosome induces a primary change in the ribosome that is essential for the subsequent proper binding of t-RNA. The CD studies have been correlated with binding studies using labelled poly(U) and t-RNA. Studies on t-RNA binding to ribosome show that the presence of octa(U) enhances this binding considerably but much less than poly(U), confirming the interaction of message with more than one site on the ribosome. Poly(U) binding was studied by nuclease removal of portions of the polymer unprotected by complexation—otherwise the polymer would be retained by filter even if unbound. $tRNA^{phe}$ enhanced the amount of U bound to ribosome either when added prior or subsequent to poly(U). Prior addition of tRNA gave a 25-fold enhancement, and subsequent addition 35-fold enhancement. Thus the symbiotic effects of message and t-RNA on their mutual binding to ribosome, as well as the importance of a proper binding sequence, deduced from CD, are confirmed by filtration assay.

B. Effects of aromatic amino acid residues of proteins on reaction with DNA. Efforts to understand the structure of chromatin, and how the chromosomal material changes with age, depend largely on understanding how proteins bind to nucleic acid in chromatin. We have carried out various types of experiments to determine what effects the aromatic amino acid residues produce in histone-like polypeptides, e.g. polypeptides containing many lysine residues. The presence of aromatic amino acids has the following effects: 1) melting is much less cooperative, 2) rewindability is reduced, and 3) the ORD and CD spectra, in contrast to those of DNA-poly(lys) complexes, are similar to those of DNA. These effects all indicate that the aromatic residues prevent the cooperative binding of the lysine, i.e., each lysine acts much more independently than in the absence of the aromatic residues. Interaction of nucleotide bases with the neutral amino acids tends to offset the protective action of lysine residues in binding to phosphate.

C. Stable and metastable structures in the reaction of DNA with polypeptides. A method was previously developed for determining whether an equilibrium complex is spontaneously produced between DNA and polypeptide. If similar ORD and CD characteristics are exhibited by complexes produced by direct mixing (DM) or gradient dialysis (GD), spontaneous generation of the equilibrium complex occurs; if they are different, metastable complexes can exist. Preparations of complexes of DNA with polypeptides containing lysine in combination with one of four other amino acids, alanine, tyrosine, phenylalanine, and serine, all produce different structures by GD and DM techniques. In sharp contrast the same complex is obtained by either DM or GD from DNA and histone I. Thus histone I differs from all the synthetic polypeptides studied in producing no metastable DNA complex.

D. The effect of metal ions on DNA-polypeptide interaction. 1. The results of many experiments on the modification of the DNA-poly(lys) reaction by divalent metal ions lead to the following conclusions: Poly(lys) has two major effects on DNA: (a) the DNA becomes aggregated and (b) the DNA is stabilized. Metal ions increase the aggregation and decrease the stabilization. 2. Metal ions counteract the effect of aromatic amino acid residues; they produce cooperative melting behavior with complexes of DNA and polypeptides containing lysine together with either phenylalanine or tyrosine.

E. The reaction of poly(lys) with selected sequence polynucleotides. The importance of nucleotide sequence and method of preparation for the structure of nucleic acid-protein complexes can be readily studied with double-stranded synthetic polynucleotides containing dA and dT bases. One of these is poly d(AT), which contains a regular sequence of alternating dA and dT on each strand; another is poly dA·poly dT, which contains only dA on one strand and only dT on the other. The poly d(AT) complex produces a complex with poly(lys) with an unusual ORD spectrum, and one single melting step. Binding of poly(lys) with poly dA.poly dT, on the other hand, results in no change whatever in the ORD spectrum of the polynucleotide. Evidently the complex produced between poly(lys) and poly d(AT) requires the alternating sequence of this polymer. This suggests that the lysines react with both phosphate and base components of the polynucleotide, and perhaps accounts for some of the specificity in DNA-histone binding.

F. Age related changes in chromatin structure. Soluble chromatin isolated from human fibroblast cells in tissue culture (WI-38 cells) has been titrated with poly(lys) in an effort to determine how the accessibility of DNA in chromatin changes with age in these cells. Experiments to date reveal no significant age changes. These results may be attributed to the fact that the chromatin in "old" and "young" cells was solubilized, and this procedure could destroy any age changes that may exist. Experiments are therefore underway to determine if age changes can be found when the chromatin structure is maintained more nearly as it exists in the undisrupted nucleus.

Significance to Bio-medical Research and to the Program of the Institute: An understanding of the structure of chromatin is required to understand the mechanism of gene regulation, which is of direct consequence to the study of development and aging. The ribosome is the locus of protein biosynthesis, and the agency or locus of much of its control. Our physical studies are designed to provide direct information about structures and interactions which are intimately involved in the synthesis process and its ribosomal control. If we are able to understand these interactions, it will then be possible to determine how they may change with age.

Proposed Course of Project: The interactions of polypeptides with DNA, and the effect of metal ions on these interactions, will be continued in an effort to understand not only the binding of protein to DNA in chromatin, but also protein-DNA interaction as in the binding of nucleic acid enzymes to DNA. We shall explore the significance of metastable states in the structure of chromatin.

Keyword Descriptors: Nucleoprotein, ribosome, nucleic acids, aging, conformational changes, messenger RNA, transfer RNA, protein synthesis, circular dichroism, DNA, metal ions, polynucleotides, chromatin.

Honors and Awards:
G. Eichhorn was invited to participate in the International Conference on Biology and the Future of Mankind, Paris, France, September, 1974.

Publications: None

Serial No. Z01 AG 00046-05 LMA
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Polymers as Biological Reagents

Previous Serial Number: HD-GMA5

Principal Investigator: J. Pitha

Other Investigators: P. Pitha
J. Smid
E. Wimmer

Cooperating Units: Johns Hopkins University, Baltimore City Hospitals
State University of New York, Syracuse
State University of New York, Stony Brook

Man Years:

Total:	1.9
Professional:	.9
Others:	1.0

Project Description:

Objectives: The project focuses on the effects of synthetic polymers on human cells grown in tissue culture and on the interaction of polymers with viral systems (animal viruses) and age-related changes in these phenomena. The main class of macromolecules studied consists of the vinyl polymers of the general formula (-CH Heterocycle - $CH_2)_n$. The ultimate objectives are to determine which synthetic polymers can be used pharmaceutically. Viral infection in senescent cells is studied to obtain clues about errors in protein synthesis.

Methods Employed: The study requires a rather broad range of chemical and biological methods. Most chemical syntheses, and the in vitro work with enzymatic systems (replicase) is done at the Gerontology Research Center. The chemical synthesis of polymers which bind cations is done by Dr. J. Smid at the State University of New York, Syracuse, N.Y. The study of the replication of murine leukemia virus is done in collaboration with Dr. P. Pitha, the study of effects of polymers on animals is done in collaboration with Dr. V. Vengris, both of the Department of Medicine, Johns Hopkins University.

Major Findings: A. Probes for errors in senescent cells. Holliday et al previously described a marked increase in concentration of altered proteins in aged cells. If this increase is due to errors in protein synthesis, challenge of these cells with virus should result in a corresponding increase in altered viral proteins. Such a challenge of WI38 cells was carried out with polio virus. No proportionate change in viral proteins was observed. It is concluded, therefore, that the Holliday alterations are not due to errors in protein synthesis. Posttranscriptional changes in the proteins can better explain all of the results.

B. Vinyl analogs of nucleic acids. Polyvinyladenine inhibits in vitro an enzyme (reverse transcriptase) which is in a crucial way involved in the replication of leukemia virus and also inhibits the actual replication of the virus in living cells in culture. These phenomena are not necessarily related as the polymer may influence other steps of viral replication than the reversed transcription - e.g. synthesis of some viral proteins or release of the virus. Study of the mechanism of inhibition of viral replication was undertaken and the point of inhibition was localized into the early stages of viral replication where the reverse transcriptase is used.

A mechanistic study of inhibition of reverse transcriptase by polyvinyladenine indicated that base pairing ability, which is a characteristic property of polynucleotides and their analogs may not be involved and thus is unnecessary in the inhibitory action. This finding may considerably enlarge the class of polymers with possible antiviral activity. To prove this hypothesis the following polymers were synthetized: poly(9-vinyl-N,N-dimethyladenine) poly(7-vinyltheophylline), poly(7-vinylpurine) and poly(9-vinylpurine). The last polymer was found to be a very potent inhibitor of reverse transcriptase and also to block replication of leukemia virus in cells in culture.

C. Ionophoric polymers. Organic compounds which complex ions of alkali metals have often unusually strong biological effects. A polymeric complexing agent was therefore studied. Polyvinylbenzo-18-crown-6, a water soluble polymer containing crown units which bind K^+, in the presence of this ion forms insoluble complexes with polyadenylate. The reaction is specific for the polymer, as in presence of corresponding concentration of benzo-18-crown-6 no precipitate is formed. Also, coprecipitation of vinyl polymer and polyadenylate occurs in presence of Na^+, Mg^{2+} or Mn^{2+}, ions which are not bound to crown units but there is nevertheless an interaction as the vinyl polymer is strongly bound to immobilized polyadenylate. Vinyl polymer strongly inhibits the reverse transcriptase activity of virions of murine leukemia and of E. coli polymerase 1. No such inhibition is observed for the monomeric benzo-18-crown-6. The inhibition is due to the interaction of vinyl polymer with polynucleotide template and is immediately reversed by the addition of excess polynucleotide.

Significance to Bio-medical Research and the Program of the Institute: Synthetic compounds of high molecular weight differ profoundly from low molecular weight compounds in their interaction with living cells. They are taken up by a different mechanism. Another difference is in their degradation in cells; synthetic macromolecules have a much longer life

span since they are not susceptible to the usual cellular degradative processes. Both these differences have practical importance only when molecular weight compounds in their interaction with living cells. They are taken up by a different mechanism. Another difference is in their degradation in cells; synthetic macromolecules have a much longer life span since they are not susceptible to the usual cellular degradative processes. Both these differences have practical importance only when polymers with a selective biological action can be designed and synthetized. Vinyl polymers here described do show some selectivity thus pointing to their potential use as pharmaceuticals.

Proposed Course of the Project: The present results show that synthetic polymers can elicit a selective biological effect, in a manner similar to that of low molecular weight pharmaceuticals. By directed synthesis and study of the basic biological effects of polymers it is hoped to gain the knowledge necessary for the design of more potent preparations.

Keyword Descriptors: Polynucleotides and their vinyl analogs, ionophoric polymers, antiviral effects, inhibitors of reverse transcriptase, senescent cells in culture, aging

Honors and Awards: None

Publications:

Pitha, J. and Scheit, K. H.: Hydrogen Bonding Ability of 2,4-Dithiouridine Derivatives. Biochemistry 14:554-558 (1975).

Pitha, P. M., Pitha, J. and Rowe, W. P.: Lack of Requirement of Reverse Transcriptase Function for the Activation of Murine Leukemia Virus of Halogenated Pyrimidines. Virology 63:568-572 (1975).

Pitha, J.: Affinity Gel Electrophoresis of Polynucleotides. Anal. Biochem., in press.

Press, G. and Pitha, J.: Aging Changes in Uptake of Polysaccharides by Human Diploid Cells in Culture. Mech. Aging Development 3:323-328 (1974).

Serial No. Z01 AG 00047-05 LMA
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Relation of Structure and Function in Hemoglobin

Previous Serial Number: HD-GMA9

Principal Investigator: J. Rifkind

Other Investigators: None

Man Years:

Total:	1.05
Professional:	.8
Others:	.25

Project Description:

Objectives: (1) To study the binding of ligands to hemoglobin, and the role of the protein in controlling this function. (2) To study the mechanisms for maintaining hemoglobin in its functional form. (3) To study the mechanisms involved in regulating the transport of oxygen to the tissues.

Methods Employed: Various preparative procedures are used to purify hemoglobin and to separate various components of the erythrocyte. Visible, uv and atomic absorption spectroscopy, as well as gel electrophoresis is used to analyze for various erythrocyte components. The oxidation of hemoglobin is investigated under various conditions with and without the addition of various substances. Binding of metal ions and other small substances are studied by equilibrium dialysis. Effects on the oxidation and oxygenation are correlated with the binding of various substances.

Major Findings: A. The effect of the conformation of hemoglobin on the rate of oxidation. Normal hemoglobin undergoes a change in conformation when oxygen or other ligands are bound. This conformational change also takes place in the blood, and is responsible for the cooperative uptake and release of oxygen. The effect of this conformational change on oxidation has been studied by investigating the rates for the Cu(II) induced oxidation at various oxygen pressures. Chemical modifications which shift the equilibrium between the "liganded" conformation and the "unliganded" conformation have also been used to elucidate the effect of this conformational change on oxidation. We have been able to demonstrate that forcing hemoglobin into the "liganded" conformation decreases the rate at which

Cu(II) oxidizes hemoglobin, but increases the rate at which Cu(I) formed by the oxidation of hemoglobin is reduced back to Cu(II) and is available to oxodize more hemoglobin. Both of these changes can be explained by a decrease in the affinity for copper, at the site involved in the oxidation, when hemoglobin is in the "liganded" conformation.

B. The effect of sulfhydryl reagents on the Cu(II) induced oxidation of hemoglobin. There is a high concentration of reduced glutathione in the erythrocyte. While reduced glutathione does not directly react with the sulfhydryl groups of hemoglobin, we have previously shown that it does react in the course of reducing oxidized hemoglobin. This reaction is thought to be significant in certain abnormal situations as well as in older organisms. We have, therefore, studied the effect of this reaction on the binding of copper to hemoglobin and the Cu(II) induced oxidation. We have shown that modifying hemoglobin with SH reagents does not affect the affinity of copper for hemoglobin. Furthermore, the rate for the regeneration of Cu(II) from Cu(I) formed by the oxidation of hemoglobin, which is limited by the dissociation of copper from the site involved in the oxidation, is relatively unaffected by this modification. However, the rate at which Cu(II) oxidizes hemoglobin is drastically reduced. These results imply that the reaction with sulfhydryls decreases the rate for the electron transfer process between Cu(II) and Fe(II).

C. Evidence for an erythrocyte protein which affects the oxidation of hemoglobin. We have previously shown that Cu(II) plays a dominant role in the oxidation of hemoglobin. Recently, we have observed that EDTA has a stabilizing affect on hemoglobin in addition to that of complexing Cu(II). This phenomenon was demonstrated by the addition of EDTA and TRIEN to hemoglobin purified with a mixed bed resin, which removes all complexable Cu(II). The complexing agent TRIEN, as expected, had no effect on the rate of autoxidation, while EDTA further stabilized the hemoglobin. Furthermore, measuring the changes in Cu(II) activity produced by adding different concentrations of EDTA to concentrated hemolyzed cells indicate that a decrease in the rate of autoxidation is produced in a region wherein the Cu(II) activity does not change.

These results can be explained by the presence of a protein present in the erythrocyte which catalyzes the oxidation of hemoglobin. The activity of this protein can be inhibited by EDTA. We find evidence for such a protein fraction by pooling the non-hemoglobin protein fractions which run off CM-Sephadex at pH 6.5 and rechromatographing this material on G-75 Sephadex.

It was found that the protein fraction with a similar molecular weight to that of hemoglobin enhances the rate of oxidation of hemoglobin. Polyacrylamide gels of various tubes in this region of the chromatogram suggest that the extent that the rate of oxidation is enhanced can be correlated with a particular protein band.

D. Changes in the ability of the erythrocyte to transport oxygen with age. Various changes in the erythrocyte of the older person have been reported. We have analyzed these changes in terms of their effect on the ability to release oxygen to the tissues. An appreciable increase with age in the

concentration of glutathione has been reported and related to an increased role in the reduction of hemoglobin. We have shown that the reduction of hemoglobin by glutathione results in a modified hemoglobin with a higher affinity for oxygen, making the release of oxygen more difficult. The resulting hypoxia should, and has been reported to, stimulate the synthesis of 2,3-DPG, which decreases the oxygen affinity of hemoglobin compensating for the hypoxia. The increase in 2,3-DPG is relatively small and may not completely compensate for the effect of the reaction with glutathione. The inability to utilize the synthesis of 2,3-DPG to fully compensate for the hypoxia can be attributed to limitations on anaerobic glycolysis resulting from reported decreases in the total concentration of nicotinamide adenine dinucleotides and in the activity of certain enzymes like aldolase.

Significance to Bio-medical Research and to the Program of the Institute: The physiological role of hemoglobin is to transport oxygen from the lungs to the cells. The efficient uptake and release of oxygen requires cooperative oxygen binding and the proper regulation of the oxygen affinity. It is also necessary to limit the oxidation of hemoglobin in order to maintain an adequate concentration of functional hemoglobin. These studies thus help to elucidate a vital function of organisms. The aging process can involve changes in the ability of the organism to transport oxygen to certain tissues.

Proposed Course of the Project: (1) We plan to continue our studies on the various factors which influence the autoxidation of hemoglobin in the erythrocyte and in purified systems. (2) Structural and kinetic studies are planned to determine the relationship between the binding of Cu(II) to hemoglobin and the oxidation of hemoglobin. Attempts will be made to determine where the Cu(II) binds and how electrons are transferred between the Cu(II) and the Fe(II). (3) We plan to study age related changes in the level of GSH, 2,3-DPG, and other erythrocyte components. We also intend to measure the concentration of hemoglobin modified by glutathione, and the ability of the erythrocyte to synthesize 2,3-DPG. These studies should enable us to relate changes in the erythrocyte to the ability of the erythrocyte to transport oxygen.

Keyword Descriptors: Hemoglobin, oxidation of hemoglobin, age related erythrocyte changes, cooperative uptake and release of oxygen, hypoxia, erythrocyte components, Cu(II) induced oxidation, conformation of hemoglobin, glutathione in the erythrocyte, binding of Cu(II) to hemoglobin

Honors and Awards:
Dr. Rifkind was invited to present a seminar to the Heme-Protein group at the National Institutes of Health, Bethesda, Maryland.

Publications: None

Serial No. Z01 AG 00048-01 LMA
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Mechanism of Metal Ion Transport Across Membranes

Previous Serial Number: NDS(1)-61-LNC/EC 813
NHLI-74

Principal Investigator: J. Froehlich

Other Investigators: R. W. Albers
R. L. Berger

Cooperating Units: NIH, NINDS, Lab. of Neurochemistry, Bethesda, Md.
NIH, NHLI, Lab. of Technical Development, Bethesda, Md.

Man Years:

Total:	1.25
Professional:	1.0
Others:	0.25

Project Description:

Objectives: (1) To understand the biochemical basis of Na^+ and K^+ transport by investigation of the kinetic effects of these ions on the energy-linked partial reactions of ATP hydrolysis, (2) to obtain information on the kinetics of Ca^{2+} accumulation by cardiac muscle microsomes, (3) to determine how Ca^{2+} transport in cardiac muscle is affected by the aging process.

Methods Employed: The interaction of metal ions with microsomal membranes obtained from eel electric organ and cardiac muscle are studied using (1) rapid chemical quenching and (2) millipore filtration. The first of these methods looks at the effects metals have on the ATP partial reactions which are coupled to ion transport. Rapid initiation and termination of the enzyme reaction is accomplished by means of a chemical-stop mixing apparatus capable of measuring reactions with half-lives of .005 seconds or larger. A tandem arrangement of mixers allows two substrates to be added in sequence before the reaction is quenched in acid. Millipore filtration which provides a direct measurement of the steady state distribution of Ca^{2+} ions is currently being evaluated as a possible tool for measuring the presteady state phase of Ca^{2+} accumulation. A high pressure filtration chamber which attaches to the chemical-stop mixing apparatus by a length of stainless tubing has been developed for this purpose. The time elapsed

between mixing and filtration is controlled by varying the tube length and speed of the motor used to drive the syringes.

Major Findings: A. Mechanism of sodium and potassium transport by (Na^+-K^+) ATPase: Phosphorylation of electric organ microsomes by ATP occurs in the presence of Na^+ and the absence of K^+. In the presence of saturating levels of K^+ approximately one-half of the phosphorylated sites are converted to a second intermediate complex which is labile in acid. Breakdown of this intermediate, designated E·P in the following scheme, is rate-limiting:

$$E + ATP \rightleftharpoons E{\cdot}ATP \xrightleftharpoons{Na^+} E \sim P \xrightleftharpoons{K^+} E{\cdot}P \rightleftharpoons E + P$$

As a function of ATP concentration the $[E \sim P]/[E{\cdot}P]$ ratio remained near unity in spite of an apparent increase in the rate of E·P decomposition. According to this scheme the increase in the rate-limiting step which results from substrate activation is expected to reduce E·P relative to $E \sim P$.

It has been proposed that (Na^+-K^+)ATPase behaves according to a 'flip-flop' or 'half-site' mechanism in which the functional unit is an enzyme dimer. In this formulation catalytic transformations in the separate halves of the dimer are conformationally coupled. Thus, it is assumed that in the presence of K^+ phosphorylation of one site is coupled to "inactivation" of its neighbor leading to accumulation of $P \sim E{\cdot}E$ and $P \sim E{\cdot}E{\cdot}S$ in the steady state. By contrast we find that dephosphorylation leads to E·P with $[E \sim P] \simeq [E{\cdot}P]$. In terms of a 'flip-flop' mechanism this behavior suggests the following sequence

$$2S + E{\cdot}E \rightleftharpoons S{\cdot}E{\cdot}E{\cdot}S \rightleftharpoons P \sim E{\cdot}E{\cdot}S \rightleftharpoons P{\cdot}E{\cdot}E \sim P \quad (S = ATP)$$

in which the appearance of E·P in one of the half units is obligatorily coupled to the formation of $E \sim P$ at the neighboring half unit.

The possibility that $E \sim P$ represents a high affinity state of the carrier for Na^+ is suggested by the fact that the formation of $E \sim P$ is activated by low concentrations of Na^+. By analogy E·P which is formed at low concentrations of K^+ ($< .1mM$) may be high affinity for K^+. Since low affinity states for both species must exist it is not unreasonable to assume that $E \sim P$ and E·P show low affinity for K^+ and Na^+ respectively. In relation to the kinetic scheme this means that half units alternate between high and low affinity for Na^+ or K^+ out of phase with each other. Thus while one unit picks up Na^+ and releases K^+ ($E \sim P$ state) its neighbor picks up K^+ and discharges Na^+ (E·P state).

B. Ca^{2+} accumulation by cardiac microsomes: An active preparation of sarcoplasmic microsome has been obtained from rat heart which is relatively free of mitochondrial contamination. This preparation shows an increased rate of Ca^{2+} accumulation in response to concentrations between .01 and 3μM and thereafter declines. An initial comparison of uptake by 6 and 24 month old rats shows that V_{max} remains constant but that K_m decreases in the older population. This apparent decrease in affinity for Ca^{2+} is consistent with an increased contraction duration observed in isolated aged rat myocardium (Lakatta et al, J. Clin. Invest. 55:61, 1975).

Significance to Bio-medical Research and to the Program of the Institute: A first step in understanding how a vital physiologic activity such as heart muscle contractibility changes with age is to understand the elementary steps of the transport processes which are involved in regulation of that function.

Proposed Course of the Project: Transient state kinetic studies of Ca^{2+} uptake using colorimetric and isotope filtration methods will be continued in an effort to identify the specific steps in the transport mechanism which are affected by aging. The partial reactions of ATP hydrolysis coupled to transport will also be investigated to evaluate possible changes related to age.

Keyword Descriptors: Transport, $(Na^{+}-K^{+})$ATPase, $(Ca^{2+}-Mg^{2+})$ATPase, transient kinetics, rapid chemical quenching, cardiac muscle, aging

Honors and Awards: None

Publications:

Froehlich, Jeffrey P. and Taylor, Edwin W.: Transient Kinetics of Sarcoplasmic Reticulum Adenosine Triphosphatase. J. Biol. Chem. 250:2013-2021 (1975).

NICHD ANNUAL REPORT
July 1, 1974 through June 30, 1975
Gerontology Research Center
Laboratory of Behavioral Sciences

The goals of the Laboratory of Behavioral Sciences are: 1) to describe in quantitative terms the behavioral changes that take place with age, to determine the basic mechanisms of these changes and to integrate these findings into the total scientific program of the Center; 2) to develop techniques and programs to mollify or eliminate the psychologically mediated impairments and disabilities of the aged. During the past year progress has been made on each of these goals.

A collaborative study between investigators in the Learning and Problem Solving Section and investigators from the Clinical Physiology Branch on the interaction of ethanol and age has been completed. These studies have shown that ethanol more severly impairs old (55-80 yrs.) than young (20-54) subjects on a number of important behavioral measures including reaction time and delayed recognition (memory). The older subjects not only were more severely impaired acutely, but also recovered significantly more slowly. These findings of an age-related response to ethanol strongly suggest that additional studies should be initiated to determine whether current blood-alcohol standards should be modified to include an age factor.

Studies from other laboratories have shown cohort as well as age effects in a variety of measures of intellectual performance. Cohort effects arise because persons born during a particular period in history will have unique experiences which are attributable to the socio-cultural milieu in which they are reared. For example, children reared during the 1930s when there was a severe depression, will have vastly different attitudes and skills than will children who were reared during the relatively affluent 1950s. A 30 year old born in the 1930s is likely to perform differently on a variety of behavioral tasks than is a 30 year old who was born in the 1950s, and these differences are cohort-related rather than age-related: Thus, longitudinal analyses of behavior must be controlled for cohort effects. Studies of longitudinal changes of memory and learning in this Laboratory have shown that there are age-related deficits in performance: Men who were 69 to 76 years at the time of first measurement showed the greatest decline in performance on retesting. Studies of groups of men with equivalent birth dates (i.e., cohorts who were tested at different ages) have shown that: The cohort sample that was older when tested performed less effectively on several tests of learning and memory; and the differences among cohorts were such that the declines in performance were greatest for the earliest born cohorts. Impairment in both longitudinal and cohort samples was similar, older subjects committed more errors on first testing; furthermore, older subjects in the longitudinal group increased their errors significantly more than did younger subjects on retesting. Therefore, whether age comparisons are made longitudinally, cross-sectionally or within cohorts, the results are the same: learning and memory decline with age, and the decline is greatest for the oldest men.

Studies of problem solving in which learning and memory factors are minimized are showing important age-environment interactions. On the one hand,

laboratory studies of age differences in concept problem solving have shown declines after age 60. These findings replicate previous cross-sectional findings in this Laboratory. On the other hand well-practiced subjects in their 60s -- i.e., subjects who have had the opportunity to solve hundreds of problems -- attained a level of performance similar to that of young adults. Furthermore, even subjects in their 90s are showing that they too can solve these difficult problems.

In animals studies now going on in the Learning and Problem Solving Section, longevity has been found to be significantly related to the factors of body weight, diet and exercise. Among rats which were not allowed to exercise, about 40% (22 of 60) failed to survive to age 22 mos. Among rats given ad-lib access to run-wheels from age 45 days, only 8% (2 of 24) were dead at 22 mos. In a study of two inbred strains of mice and their hybrid cross, animals which received a low (4%) protein diet showed a greater degree of survival than did animals which received a normal (26%) protein diet: The percentages of mice which reached advanced old age (based on strain-specific norms) on low protein diets were 34% for one strain, 26% for the other strain and 42% for the hybrids; on normal protein diets survival was 10%, 4% and 10% respectively. Among five genetically identifiable groups of mice, the non-mutant control animals (C57BL/6J) lived about 14% longer than did two light weight mutant groups, and about 35% longer than did two very heavy mutant groups; nevertheless, the higher the peak weight among individual animals the longer they survived relative to their group expectancy. Furthermore, this last study also showed that animals with the longest growth period -- i.e., the greatest age of attainment of peak weight relative to their norms -- survived longest.

Collaborative studies between the Psychophysiology Section and the Gastro-enterology Unit of the Baltimore City Hospitals have now shown conclusively that a significant number of patients with chronic, severe fecal incontinence associated with organic lesions can be trained to become continent. It would be important now to evaluate the applicability of this procedure to older, incontinent patients. In addition to the studies on patients with gastro-intestinal disorders, this Laboratory also is studying patients with high blood pressure. Studies designed to describe the hemodynamic correlates of learned blood pressure control in greater detail have just begun. As part of this project, a study of forearm blood flow is being carried out in which flow is measured serially over two min. at 10 sec. intervals by means of a mercury-in-rubber strain gage plethysmograph. When this study was done a number of years ago using a water, venous-occlusion plethysmograph, it was shown that there is biphasic pattern of flow characterized by an initial increase followed by a decrease and then a return to baseline. Present studies suggest that the biphasic pattern, if it exists at all, is greatly attenuated when flow is measured with a mercury-in-rubber gage.

In a continuing program designed to identify the physiological and behavioral mechanisms of learned cardiac control, two studies of such learning have been completed using monkeys as subjects. In one study of the physiological mechanisms of such learning, it was shown that animals have characteristic heart rates below which they can not slow: The relationship between degree of slowing (change in heart rate from baseline) and baseline heart

rate is linear. When the animals are given the vagolytic drug, atropine, their ability to slow heart rate (as measured by the line of best fit relating change in heart rate to baseline heart rate during nondrug sessions) is reduced, however, they still are able to slow. When the animals are given the sympatholytic drug, propranolol, the ability to raise heart rate is enhanced. Behavioral studies of animals have shown that when animals are required to speed their heart, they tend to increase general activity whereas when they are required to slow heart rate they tend to decrease activity. Nevertheless, within any particular animal, the ability to slow or to speed its heart is uncorrelated with activity. Thus, animals apparently establish "sets" to speed or to slow heart rate, however, once set, the control of heart rate appears to operate independently of body movement.

In addition to the research programs described above, the Laboratory also serves as a resource for non-intramural investigators who are conducting basic research in aging. This year a graduate student from the University of Delaware, Ms. Teena Wax is completing her doctoral dissertation entitled, "Behavioral patterns of activity and self-selection of lighting as a function of age, strain and illumination intensity."

Project No. Z01 AG 00061-13 LBS
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Behavioral genetics and aging

Previous Serial Number: HD-LBS-1

Principal Investigator: Charles L. Goodrick, Ph.D. (50%)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	1.15
Professional:	.50
Others:	.65

Objectives: The principal objectives of this project are (1) to determine group differences for behavioral traits and longevity among inbred strains of mice; (2) to determine heritability (degree of genetic determination vs. degree of environmental determination), mode of inheritance (e.g., over-dominant, dominant, intermediate, or recessive), and number of segregating units (gene blocks) controlling a particular trait (e.g., longevity); and (3) to examine relative behavioral differences among mouse strains as aging progresses. Other objectives include determining the influence of diet (e.g., protein available) on behavioral traits, growth, and longevity; and identifying single-gene influences upon behavioral traits, growth, and longevity.

Methods Employed: Inbred mice (C57BL/6J and A/J) of a high degree of homozygosity are maintained under uniform environmental conditions. The animals are tested behaviorally during one period of their life span, viz, when mature, mature-old, or aged. Old age is determined as the 50% mortality point for groups maintained throughout their life span. Statistically reliable techniques have been developed to determine behaviors relevant to natural selection such as exploration, general activity level, emotionality, simple or complex problem solving ability and taste preference. The use of segregating F_2 hybrid groups allows an estimate of the mode of inheritance, e.g., dominant or intermediate, and the number of gene blocks or segregating units controlling behavioral traits or life span. For studies in which protein intake is varied for groups of inbred and hybrid mice, isocaloric synthetic diets are used. Deprived animals receive 4% casein in their diets, whereas the control group receives a 26% casein diet. Numerous kinds of mutant mice are maintained on the C5BL/6J background at the Jackson Memorial Laboratories; Bar Harbor, Maine. Our work has concentrated on the albino, beige, yellow and obese mutations.

Major Findings: A. Human and mouse populations show an increment in body weight following birth, to some maximum value later in the life span, normally followed by a decline in weight toward the end of the life span. McCay, in important early gerontological studies, suggested that there was a positive relationship between duration of growth and longevity. For the mouse, populations exist which differ at one genetic locus and which also attain an unusually high maximal body weight, viz, the obese mouse and the yellow mouse. These mouse groups also may differ in average longevity, i.e., have shorter life spans, compared with nonobese control mice. Such populations are useful in research because of the maximal control of genetic constitution. The purpose of the present experiment was to determine changes in body weight from just after weaning until death for mutant mouse groups which differ in body weight and for a nonmutant control group, to examine the relations of growth and maximal body weight to longevity. Body weights were obtained monthly for mutant groups which differ in body weight (bg, c^J, A^y, ob), and for a control group (C57BL/6J) (n = 16, N = 80). The mean longevity was significantly lower for all mutant groups compared with the mean longevity of the control group. Although obese mutant mice had a shorter life span than other mutant groups, mice which attained an equally high body weight (yellow, A^y) did not differ significantly in longevity from thin mutant mice (bg, c^J). Moreover, maximum body weight was positively correlated with longevity for all mouse groups. All mouse groups showed a terminal decline in body weight at the last stage in the life span, except the albino group which continued to grow. In general, terminal weight loss was greater for long-lived mice than short-lived mice. Within each group there was a positive relationship between growth duration (the length of the weight increment phase of the life span) and longevity.

B. Mice of the A/J (N = 100) and C57BL/6J (N = 100) strains and an F_1 hybrid group (N = 100) were subjects. The A/J strain and F_1 hybrid group tolerated the low protein diet well, while the C57BL/6J strain had a large number of early deaths for groups fed low protein diets. Of even greater interest is the higher number of animals (within each genetic group) which reached advanced old age when fed low protein diets compared with mice fed diets of normal protein.

C. Measures of life span were obtained for male and female A/J, BALB/cJ, C57BL/6J and DBA/2J inbred mouse strains and the six possible hybrid combinations (N = 500, 10 groups, 25 male and 25 female per group). C57BL/6J mice were long lived while A/J, BALB/cJ, and DBA/2J mice were short lived, with the exception of female BALB/cJ mice which lived as long as C57BL/6J mice. Female BALB/cJ and two female hybrid mouse groups with a BALB/cJ parent lived longer than males, but significant sex differences were not obtained for other groups. In general, the mode of inheritance of longevity was overdominant. A second study (N = 400) of the longevity of A/J and C57BL/6J strains, F_1 and F_2 hybrids obtained significant group differences, but the mean longevities were not significantly different from those obtained in the first study. C57BL/6J mice were significantly longer lived than A/J mice, sex differences in longevity were not obtained, and the hybrid F_1 group lived significantly longer than the longest lived parental group. It was estimated that there was one genetic factor (K, gene locus) associated with longevity and the coefficient of genetic determination for longevity was estimated as between .48 and .79.

Significance to Bio-Medical Research and Program for the Institute: The study of the genetics of behavior and longevity allows an assessment of: (1) the mode of inheritance (i.e., dominant, intermediate, etc.) for the factor studied (2) the relative importance of hereditary and environmental factors; and (3) the number of genes or gene blocks which control the factor studied. Lack of adequate dietary protein is a condition which affects a large proportion of the world populations. This project attempts to determine the effect of diet (such as different proportions of protein in the total diet) during particular stages of the life span upon behavior and longevity for animal populations which differ in genetic constitution. Studies of single gene mutant animals are of importance because they allow the assessment of the importance of a specific genetic locus for physiological or behavioral factors.

Proposed Course of Project: Further inbred strains and F_1 hybrid groups are being studied to determine the generality of mode of inheritance for behavioral factors. Cross-sectional and longitudinal studies of mouse behavior will continue with mouse strains. The longevity of inbred and hybrid groups are also being determined. Experiments with low and normal protein diets should determine: (1) the relationships between growth rate and longevity, (2) the effect of low, normal, or high protein diets upon behavior at maturity after access to these diets during various stages of development, and (3) the effects of a diet of low, normal or high protein at the time of measurement upon behavior.

Keyword Descriptors: behavioral genetics, aging, mouse.

Honors and Awards: Ms. Teena Wax presented a paper entitled "Light preference of albino and pigmented mice as a function of age and illumin-mination intensity" at the annual meeting of the American Psychological Association, September, 1974.

Publications: Goodrick, C. The effect of protein malnourishment and caging on growth and behavior of laboratory mice. Developmental Psychobiology, 1974, 7, 249-256.

Goodrick, C. Life span and the inheritance of longevity of inbred mice. Journal of Gerontology, In Press.

Project No. Z01 AG 00062-02 LBS
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Daydreaming and Aging: Normative and Experimental

Previous Serial Number: HD-LBS-2

Principal Investigator: Leonard M. Giambra, Ph.D. (40%)

Other Investigators: Thomas Traynor, M.A.

Cooperating Units: Baltimore City Hospitals
Towson State College
Morgan State College
College of Notre Dame of Maryland
Miami University of Ohio

Man Years:

Total:	.60
Professional:	.40
Others:	.20

Project Description:

Objectives: The goals are: (1) to determine the incidence and content of daydreaming in specific subpopulations (e.g., young, middle aged, elderly) from various socio-economic classes, various races, etc; (to attempt to relate these differences in daydreaming to any underlying mechanisms such as physiological state, education, cultural values and beliefs, differential daily experiences, and (3) to investigate experimentally variables which normative studies have indicated may be potent determiners of daydreaming.

Methods Employed: The normative aspects of daydreaming are determined through the use of structured self-report. Each participant completes a 21 item biographical questionnaire and a 344 item Imaginal Processes Inventory (IPI) which has both specific and general items concerning daydreams, night dreams, fantasy, etc. To date approximately 1200 individuals from a wide variety of subpopulations have completed the IPI and bio-questionnaire. There are 28 scales in the IPI. Each item has five choices which are points on a continuum implying frequency or quantity. The choices were assigned values of 0, 1, 2, 3, or 4.

Major Findings:

I. Daydreaming as a function of age, sex, and race.

A second sample of males 24 to 91 years has been given the IPI. A first sample of women 17-91 years is currently being given the IPI and Biographical Questionnaire. This sample is incomplete for age groups 45-64 and 75-91. A first sample of blacks 17-23 years is currently being given the IPI and bio-questionnaire. This sample is also incomplete; data collection is continuing. This sample will provide black-white comparisons in this age range.

II. The time course of spontaneous thought intrusions (daydreams).

Subjects are given fifteen minutes to solve a difficult word puzzle problem. During the next 12 to 72 hours they make entries in a structured log every-time they think about the puzzle in any way. The purpose of the study is to determine the circumstances under which spontaneous thought intrusions occur for a specific "current concern" of the individual, the content of those intrusions, and the circumstances which terminate them. The log also will provide information on the time course of those spontaneous intrusions. A trial run on one individual has indicated that this approach is practicable and shown where the structured log could be improved.

III. The relationship of daydreaming and depressive affect.

Three groups of participants (male and female college students, male prison inmates) were given three self-report depression scales; Beck Depression Inventory, Zung Self-Rating Depression Scale, and Depression Adjective Check Lists in addition to the IPI. Multiple regression analyses, using three depression scale scores as "predictors" of each daydreaming scale were computed for the three subject groups. Specific hypotheses concerning relationships to depression were made on 15 of the 28 daydream scales. Overall, 8 of the 15 hypotheses were statistically significant for one or more of the three subject groups, with correlations favoring the hypothesis for the remaining groups; four of the remaining hypotheses were not statistically significant, but the data were in the predicted direction; two of the hypotheses were not supported by any group; and for one hypothesis the data were in the opposite direction.

Negative affect appears to play a major role in specific content, structure, and functional aspects of daydreams, and the degree of the influence has been quantified. The differences in daydreaming patterns between male and female subject groups were highly significant. For both the overall and individual analyses, the differences between the college and prison groups were small. This result, however, warrants considerable caution and needs to be replicated. Only the college groups are discussed below.

The differing patterns between males and females suggest that negative affect influences primarily the structural aspects of fantasy for the females, especially the frequency of daydreaming, while the primary influence of negative affect for males was on attitudinal and content parameters. This structural vs. attitudinal dichotomy suggests that females may "take refuge" from negative affect in their daydreams, but males do not and become increasingly troubled by their fantasies as negative affect increases.

Significance to Bio-Medical Research and Program of the Institute: The study of daydreaming is fundamentally a study of thought processes. In order to understand fully the thought processes of man, the total spectrum of those processes needs to be examined. In addition, it is important to know how this wide spectrum is affected by aging. Thus the study of daydreaming in adults, along with other variables, such as differences in age, socio-economic status, attitudes, etc., may help us understand the fundamental processes which underlie all these behaviors.

Proposed Course of Project:

1. Intraindividual age changes in daydreaming is currently studied in the Baltimore Longitudinal Study.

2. The relationships of biological, social, and personality characteristics to daydreaming will be explored.

3. Data on females older than college age are currently being collected.

4. Experimental studies of the time course of daydreaming are about to begin.

Keyword Descriptors:
Daydreams, intuition, depression, sex differences, spontaneous thought intrusions.

Honors and Awards: Dr. Giambra presented a paper at the American Psychological Association convention in August, 1974 entitled "Eye Movements as a function of rate in thought content and attention."

Dr. Giambra was an invited participant in a conference on structural learning under the sponsorship of the Structural Learning Society (April, 1975).

Dr. Giambra was invited to participate in a symposium at the Maryland Psychological Association Convention April, 1975. His presentation was entitled "Daydreaming from 17 to 91 Years."

Publications:

Fiester, A. R., Mahrer, A. R., Giambra, L. M., & D. W. Shaping a clinic population: The dropout problem reconsidered. Community Mental Health Journal, 1974, 10, 173-179.

Giambra, L. M. Daydreaming across the life span: late adolescent to senior citizen. International Journal of Aging and Human Development, 1974, 5, 115-140.

Ruth, J. S., & Giambra, L. M. Eyemovements as a function of attention and rate of change in thought content. Perceptual and Motor Skills, 1974, 39, 475-480.

Giambra, L. M. Daydreams-The backburner of the mind. Psychology Today. 1974, 8, 66-68.

Project No. Z01 AG 00063-08 LBS
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Psychophysiology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Learned Modification of Visceral Function in Animals

Previous Serial Number: HD-LSB-3

Principal Investigator: Bernard T. Engel, Ph.D. (50%)

Other Investigators: Sheldon H. Gottlieb, M.D. (90%)

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	3.70
Professional:	1.40
Other:	2.30

Project Description:

Objectives:

To determine the physiological and behavioral mechanisms associated with conditioned control of cardiovascular functions in the rhesus monkey.

Methods Employed:

A. Pharmacological studies

A catheter was placed in the aorta via the external iliac artery, and the animals were restrained in a primate chair contained in a sound-proofed booth. Arterial pressure was measured directly, and the differentiated pressure pulse was used to give heart rate. Following initial shaping sessions, animals were trained until they could reliably slow or speed heart rate in response to a discriminative cue. Forty sessions of alternating slowing and speeding sessions provided control data. These were followed by 12 slowing and 12 speeding sessions during which autonomic blocking drugs (methylatropine bromide, 1 mg/kg, and l-propranolol 1 mg/kg) were used. Each session consisted of a 20 minute equilibration period during which the animal sat undisturbed in a closed booth; then on drug-days, the drug was infused, on control days saline was infused; then an 8 minute baseline was obtained; finally, there was a 35 minute training session. There were four sessions/day,

B. Activity and Heart rate

Four monkeys were studied. The sound-proofed booths were fitted with one-way wide-angle observation peep-holes so that the upper part of the monkey's body

could be observed during conditioning sessions. A behavioral index was prepared by devising operational measures of gross motor activity and attention. The behavior observed included gross movement of the arms and body, vocalization, eyes opened or closed, and staring at the cue lights.

Major Findings:

A. Learned control of heart rate in monkeys

1. Pharmacological Studies

During slowing control sessions all animals showed a highly significant negative regression of heart rate change on baseline. The regression line could be extrapolated to obtain the minimum baseline heart rate below which the animal could not be expected to slow. Two of the animals showed a similar regression of heart rate change against baseline during speeding control sessions. However, there was no correlation in the remaining 3 animals. Most of the decrease in HR during slowing occurred within 4 minutes of onset of the session. Most of the increase in HR during speeding occurred within 6 minutes of onset of the session. During propranolol blockade, all animals could slow their heart rate for the first 2-4 minutes. However, only 3 animals could sustain this response. In these animals the regression of heart rate change on baseline heart rate was steeper than during control slowing. All animals could speed under propranolol blockade. Three animals showed a regression of heart rate change on baseline. All animals were able to slow during atropine blockade. However, the heart rate changes were only 10-50% of the change expected at the observed baseline heart rates during control sessions. During a session heart rate initially decreased, but then increased slightly. Correlation of heart rate change with baseline was nearly zero. Following combined sympathetic and parasympathetic blockade, all animals showed small insignificant changes in heart rate.

We conclude that during conditioned heart rate slowing there is an increase in vagal tone, and a decrease in sympathetic tone. The increased slope of the regression line of heart rate change on baseline following propranolol blockade relative to the undrugged state, suggests that the increase in vagal tone is virtually maximal as soon as the animal begins slowing. The amount of slowing during atropine blockade is less than expected and occurs during the later part of the session, suggesting a gradual withdrawal of sympathetic tone. Changes in autonomic tone during conditioned speeding were variable between animals. The delay in speeding following atropine blockade suggests a gradual increase in sympathetic tone. However, all animals were able to speed following propranolol blockade, and speeding occurred early in the session, suggesting that during speeding there was a prompt decrease in vagal tone followed by a gradual rise in sympathetic tone.

2. Activity and heart rate

During slowing training the activity was less than during baseline and during speeding training the activity was greater than during baseline. However, the correlations between heart rate and activity within the training periods were low. Although there was some overlap in heart rate level between slowing and speeding the activity scores between slowing and speeding were significantly different. We conclude that there are distinctive behavioral

"sets" during conditioned slowing and speeding. However, the lack of overlap of activity scores despite occasional overlap of heart rate changes during slowing and speeding and the low correlations between heart rate and activity suggest that distinctive behavioral sets accompany but do not cause the observed heart rate changes.

Significance to Biomedical Research and the Program of the Institute:

These studies will help us to understand the cardiovascular mechanisms of heart rate control. This knowledge will enable us to develop more effective training techniques not only for monkeys but also humans, and this knowledge will help in understanding neurocardiovascular integration.

Proposed Course of Project:

A. Learned control of heart rate in monkeys.

The findings from parts 1 & 2 are being prepared for publication. We are planning to attempt to directly condition stroke volume.

Keyword Descriptors: Monkeys, Heart rate, Blood pressure, Operant, Biofeedback, Propranolol, Atropine.

Honors and Awards:

Dr. Engel conducted the psychiatry staff conference, University of Chicago School of Medicine (October, 1974).

Dr. Engel participated in a symposium on future directions in psychophysiology at the annual meeting of the Society for Psychophysiological Research (October, 1974).

Dr. Engel guest lectured in a course on behavioral biology, University of Missouri School of Medicine (January, 1975).

Dr. Engel guest lectured in a course in bahavioral sciences, University of Maryland School of Medicine (April, 1975).

Publications:

Engel, B. T. Electroencephalographic and blood pressure correlates of operantly conditioned heart rate in the restrained monkey. Pavlovian Journal of Biological Science, 1974, 9, 222-232.

Kristt, D. A. Diencephalic vasodepressor responses independent of heart rate changes, Life Sciences, 1975, 16, 395-402.

Kristt, D. A. Vasodepressor responses evoked from rat diencephalon: Effect of pre-stimulation blood pressure. Brain Research, in press.

Kristt, D. A., Rosenberg, K. A. and Engel, B. T. Effect of prolonged intra-arterial catheterization on arterial wall. Johns Hopkins Medical Journal, 1974, 135, 1-8.

Project No. Z01 AG 00064-14 LBS
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Learning and Problem Solving Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Problem Solving and Aging

Previous Serial Number: HD-LBS-4

Principal Investigators: David Arenberg 30%
Leonard Giambra 60%

Cooperating Units: Baltimore City Hospitals
Miami University of Ohio
Towson State

Man Years:

Total:	2.65
Professional:	0.90
Others:	1.75

Project Description:

Objectives: The general goals are to explore and identify reasoning processes in man, to determine in what ways these processes change with age, and to develop techniques for reducing age deficits in reasoning performance. In this project, reasoning is studied by using problem solving procedures in which on-going solution behavior can be observed and quantified. Experiments are designed to answer such questions as: (1) Is effectiveness in acquiring relevant information affected by aging? (2) Is effectiveness in synthesizing available information affected by aging? (3) What kinds of solution strategies are used and in what ways are they related to age? (4) How does imposing a memory load affect solution strategies for young and old adults?

Methods Employed: Experiment V is a longitudinal study of concept identification. Each subject attempts to solve twelve concept problems, six types of problems which are repeated. In each problem, the subject selects instances and is informed whether his choice is or is not an example of the concept. Each selection has potential information gain which can be quantified. The primary dependent measure is the mean potential information gain for all the selections in each problem. The experiment was designed to study age differences and ages changes in different problem types and the effects of varying amount of initial information gain.

Experiment XI is a concept study in which each subject solves a large number of problems. The literature in concept identification is based almost entirely on mean effects of subsamples in which each subject solves one or a few problems. It has not been established that the variables which affect

the mean performance of groups would affect an individual in the same way. When a subject solves one or a few problems, his approach to reasoning can be characterized as unstable, variable, transient, and highly subject to chance occurrences. Solving many problems is expected to result in a steady state of performance. In that steady state, strategies can be more readily elicited and the effects of various independent variables on changes in strategy and other performance measures can be studied. After subjects solve 48 or 96 complete learning problems, they solve 8 or 16 problems in which they are asked to "think out loud" throughout each problem. The protocols of these "thinking-out-loud" problems are the primary data of this study. They are used to construct individual models of how a subject solves complex concept problems with which he is highly practiced.

In Experiment XII, five concept attainment studies were carried out. The selection procedure was used; i.e., subjects selected each instance after the first. The effect of initial instance was studied comparing: (a) the attribute identification and complete learning tasks, (b) two criteria of success, (c) different amounts of experience with each type of initial instance, (d) a history of all-exemplar or all-nonexemplar initial instance, (e) one or two irrelevant dimensions, and (f) two or three values for each dimension of the stimulus universe. Furthermore, the effects of certain interactions of the variables of (a) to (f) were investigated. The generality of any effects across concept rules was ascertained by using the affirmation, negation, conjunction, alternate denial, inclusive disjunction, joint denial, conditional, exclusion, biconditional, and exclusive disjunction rules.

Experiment XIII is a study of concept problem solving in which the primary variable is the designation label. It is assumed in the concept literature that such labels are irrelevant, but the results of Experiment X make that assumption untenable. In this study, directional labels (A and not-A) were used with the concept rules conjunction, alternate denial, inclusive disjunction, and joint denial; neutral labels (* and ⊕) were used with the concept rule-pairs conjunction/alternate denial and inclusive disjunction/joint denial. With neutral labels, rule pairs, such as conjunction/alternate denial, are indistinguishable.

Major Findings: Insufficient data are available for preliminary analyses of change in Experiment V, but recent first-time data provide an opportunity to replicate cross-sectional results based on data collected prior to 1970. Both samples were restricted to men with a college degree. The results of the replication were quite similar to the previous results. Age differences were found for all twelve problems; effectiveness measures were consistently lowest for the groups in their 60s and 70s. Under conditions of high initial information gain, effectiveness in solving problems was greater for disjunctive problems than for logically equivalent conjunctive problems for all age groups.

Thus far in Experiment XI, two young men, a 63 year-old man, and a 66 year-old woman have solved the initial set of problems and have reached the "thinking-out-loud" problems. Recently three new participants 89, 90, and 96 years of age have begun solving the initial set. Although the model is not complete, extensive progress has been made for the 63 year-old man.

In Experiment XII, the frequency of selecting negative stimulus instances was affected by all of the independent variables and several of their interactions. Negative stimulus instances were selected more frequently for: (a) positive initial instances, (b) the attribute identification task, (c) stating the concept (Rule Criterion), (d) few irrelevant dimensions, and (e) previous experience of all-positive initial instances. These effects and their interactions are likely to result from subjects' awareness of the salient variables and their attempts to deal with them. Models of concept problem solving need to take this awareness into account.

In Experiment XIII, for the four concept-rule groups which had directional labels, the means for the trial number of last error were: conjunction, 12.5; alternate denial, 44.1; inclusive disjunction, 69.9; and joint denial, 78.3. For groups with neutral labels, those who received the conjunction/alternate denial complementary rule pair had a mean of 35.6; and inclusive disjunction/joint denial, 72.6. The evidence showed that, in a complete learning task, neutral labels (a) did not make the conjunctive and inclusive disjunctive rules equally difficult in a stimulus universe of ternary-valued dimensions, and (b) resulted in problems intermediate in difficulty between the difficulties of the complementary rules with directional labels.

Significance to Bio-Medical Research and the Program of the Institute: Reasoning is among the most prized behaviors of man and among the most elusive for experimental study. In this project, methods have been and will be developed to obtain quantifiable measures of step-by-step performance on reasoning problems. Some of these methods also provide patterns of response which represent strategies in solving such problems.

Measures are obtained in current experiments to study changes in reasoning processes with age. These studies, in addition to identifying basic reasoning processes, should indicate the pervasiveness of reasoning deficits with age, whether education and cognitive activity mitigate such deficits, and what techniques could be used to minimize decline in reasoning.

Proposed Course of Project: Longitudinal data collection should provide preliminary results of conventional repeated-measures of change in the coming year and may also permit longitudinal, within-cohort age comparisons. Models for the two young and five old participants in the long-term, multi-problem study will be developed.

Keyword Descriptors: aging, problem solving, concept learning, reasoning, information processing-complex, concept identification.

Honors and Awards: Dr. Arenberg was invited to participate in a symposium entitled "The Ecology of Psychobiology, Motor, and Cognitive Performance of Older People" at the annual meeting of the American Psychological Association.

Publications: Arenberg, D. A longitudinal study of problem solving in adults. Journal of Gerontology, 1974, 29, 650-658.

Giambra, L. M. Altering the concept learner's desire for non-exemplars of the concept: the effect of nonexemplar initial instances. Journal of General Psychology, (In Press).

Giambra, L. M. Response category labels: Do neutral labels make conjunctive and inclusive disjunctive concepts equally difficult? Psychological Reports, 1974, 35, 1155-1159.

Project No. Z01 AG 00065-15 LBS
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Learning and Problem Solving Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Verbal Learning and Age

Previous Serial Number: HD-LBS-5

Principal Investigator: David Arenberg, Ph.D. (60%)

Other Investigators: Elizabeth Anne Robertson, Ph.D. (90%)
Phillip Thorne (10%)
Robert Vestal, M.D.
Carol Hausman, U. of Md.

Cooperating Units: Baltimore City Hospital

Man Years:

Total	3.40
Professional:	1.60
Others:	1.80

Project Description:

Objectives: General objectives are: (1) to identify the psychological mechanisms of age differences in acquisition and retention of verbal material in man; and (2) to identify conditions which are particularly beneficial to learning by the old so that practical methods can be developed for training the elderly.

Methods Employed: Experiments I and II are both rote learning studies which are part of the longitudinal program. Experiment I is a serial learning procedure, and Experiment II is a paired-associate learning procedure.

Experiments Ia and IIa are cross-sectional studies of serial and paired-associate learning designed to determine whether there are list differences and effects of experience for materials used in Experiments I and II.

Experiment XXVII is a study of dichotic listening and aging. A dichotic pair consists of two different digits simultaneously presented one to each ear. In addition to the dichotic messages, mixed message (in which each digit is presented to each ear) also are used.

Experiment XXVIII is a collaborative study (with the Metabolism Section of the Clinical Physiology Branch) of the effects of ethanol and aging on several memory tasks and response speed. Measures include immediate free recall, delayed free recall, delayed recognition, memory span, dichotic listening, simple reaction time, and choice reaction time at four levels of complexity. Adult age is a variable.

Experiment XXX is a study of the effectiveness of a mnemonic device on learning by the old. The procedure is to learn a "trip" through the learner's living quarters. Most mnemonics require substantial practice to overlearn the procedure in order to use it, and would probably not be suitable for the old. It is expected that because the locations are part of their everyday living they will be readily learned by old men and women.

Major Findings: In Experiment I and II, both verbal learning studies, two kinds of longitudinal analyses were included--conventional and independent samples from the same cohort. The results were similar for both paces in both studies. Mean changes were small or even positive (showed improvement) for the youngest groups, but the oldest groups (over 65) showed the largest deficits. These data show that performance of the oldest men declines most whether changes are measured within subjects, or between subjects born in the same period but measured at different times.

In Experiments Ia and IIa, the effects of practice and differences between lists used in Experiments I and II were studied. The results confirmed what was suspected from the longitudinal data. List II (used for the second-time longitudinal measure) was found to be more difficult than List I in paired-associate learning. As a result, age changes as measured by conventional (repeated measures) longitudinal analyses are over-estimated in Experiment II. Prior exposure (practice) effects were found in serial learning. As a result, age changes measured by conventional longitudinal analyses are under-estimated in Experiment I.

In Experiment XXVII, lists of four and six digits are presented dichotically (half the digits to each ear) and mixed (all the digits to each ear). Contrary to an assertion in the literature, for correctly recalled items, the incidence of sequential responding was higher for dichotic than for mixed lists.

In Experiment XXVIII, the effects of ethanol on memory, response speed, and perception are studied in old and young adults. Simple reaction time, free recall, recognition memory, and dichotic listening all declined and then improved as the ethanol was infused and then metabolized. For simple reaction time, every subject (N = 40) slowed and then increased his speed; but the old, who were initially slower, were more affected by the ethanol than the young. Every subject declined in delayed free recall. Delayed recogniton was affected little by ethanol for the young, but substantially for the old. Without alcohol, young subjects rarely have difficulty reporting single pairs of dichotically presented digits, but some old subjects do. During infusion, performance declined for 17 of the 20 old subjects and 10 of the 20 young subjects. Immediately after infusion, 13 of the old subjects but only 3 of the young recalled fewer pairs than prior to infusion.

Experiment XXX is a study to determine whether a mnemonic procedure can be learned and applied by the old to recall word lists. Preliminary results indicate that not only can the old learn the procedure readily, but they can use it to recall many of the words of a 16-word list presented once.

Significance to Bio-Medical Research and the Program of the Institute: Learning is more central to experimental psychology than any other behavior, and some of the most striking and consistently reported behavioral age differences in the gerontological literature have been found in verbal learning performance. The experiments in this project are designed to

identify basic mechanisms of learning and retention and to measure differences and changes in these function that occur with age. In additon, knowledge about experimental variables which affect age differences will be valuable in developing techniques for optimizing learning of the older person.

Proposed Course of Project: A dichotic listening study has been designed to estimate the time required by an individual to shift his attention from one information channel (an ear) to another (the other ear). The time between onsets required to identify both stimuli, an index of time to shift attention, will be studied in relation to EEG changes as well as to behavioral factors.

Keyword Descriptors: Verbal learning, memory, aging, dichotic listening, ethanol.

Honors and Awards:
Dr. Arenberg was elected president of the Division on Adult Development and Aging, American Psychological Association, for 1975-1976.

Dr. Arenberg was invited to present a seminar at the Center for the Study of Aging, Duke University Medical Center, February, 1975.

Dr. Arenberg was invited to chair a symposium entitled "Cognitive Performance Across the Adult Life-Span: A Report from the Learning and Problem Solving Section of the Gerontology Research Center" at the annual meeting of the Maryland Psychological Association, April, 1975.

Dr. Robertson was invited to participate in a symposium at the annual meeting of the Maryland Psychological Association, April, 1975. Her presentation was entitled "The Effects of Ethanol on Age Differences in Performance."

Dr. Robertson was invited to present a seminar to the Psychology Department at Notre Dame University.

Dr. Robertson presented a paper entitled "Age Differences in the Perception of Dichotic Digit Pairs" at the annual meeting of the Gerontological Society, October, 1974.

Dr. Robertson presented a paper entitled "Age Differences in Memory Performance Following Ethanol Infusion" at the Tenth International Congress of Gerontology, June, 1975.

Drs. Arenberg and Robertson were invited to write an article on aging, general learning, and problem solving for the International Encyclopedia of Neurology, Psychiatry, Psychoanalysis, and Psychology, edited by B. B. Wolman.

Publications: None

Project No. Z01 AG 00066-14 LBS
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Learning and Problem Solving Section
4. Baltimore, Maryland 21224

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Perceptual Retention and Age

Previous Serial Number: HD-LBS-6

Principal Investigator: David Arenberg, Ph.D. (10%)

Other Investigators: Elizabeth A. Robertson, Ph.D. (10%)
Phillip Thorne

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	.30
Professional:	.20
Others:	.10

Project Description:

Objectives: One general objective is to investigate the effects of interference in perceptual retention and in perception: (1) to determine whether aging results in increased susceptibility to interference: (2) to explore conditions which affect age differences in interference; and (3) to develop procedures for testing mechanisms which may account for the empirical findings.

Another objective is to study non-verbal memory and the conditions which improve such memory, especially for the old.

Methods Employed: Experiment VII is a longitudinal study of memory for designs in which subjects attempt to reproduce visual designs from memory. The Benton Visual Retention Test is used, and the primary dependent measure is the total number of errors in all ten designs. Each design consists of geometric figures presented for ten seconds and then withdrawn. The task is to reproduce each design from memory. Subjects may take as much time as they need to draw the design.

Experiment VIII is a study of age and the psychological refractory period. When two signals are presented close together in time and the task is to respond to the second, the reaction time increases as the interval between signals decreases (over the range of 500 to 50 msec). The additional time required is known as the psychological refractory period. One explanation is based on a model involving a single channel of limited capacity which processes information sequentially. When two signals occur successively at

a fast rate, the system cannot attend to and process both. As a result, the second signal must be stored until the previous signal has been processed. It has been hypothesized that the refractory period (the delay) is related to the dominant alpha period of the EEG. If so, the refractory period should be longer for old than young adults; and, furthermore, it should be related to the period of the EEG alpha. A study has been planned to compare the refractory period of young and old adults, and to correlate refractory period with alpha as measured by the EEG in the Longitudinal Program.

Major Findings; In Experiment VII, memory for designs was studied longitudinally; and, as in the verbal learning studies, both conventional and independent-samples analyses were included. The conventional analyses were based upon subjects measured initially between 1960 and 1964 and measured again six (or more) years later. The independent samples analyses were based upon first time measures between 1960 and 1964 compared with first time measures between 1968 and 1973 of men born in the same periods. The results were essentially the same for both types of comparisons. Age declines were smallest for the youngest groups, intermediate for the middle-aged groups, and largest for the oldest groups. Not only were the age changes similar for conventional and independent-samples analyses, but both longitudinal curves were similar to the cross-sectional curves of age differences of the early and the late samples. Furthermore, a replication of the conventional longitudinal analysis was carried out with the data for men initially measured between 1965 and 1967 and who were measured again between 1971 and 1974. The results of the replication were quite similar to those of the first study. Just as before, declines increased with age.

Experiment VIII is an aging study of the psychological refractory period. Tapes with pairs of signals recorded at specific intervals have been obtained, and data collection will begin as soon as the apparatus is assembled.

Significance to Bio-Medical Research and the Program of the Institute: The general idea that a person becomes more susceptible to interference as he grows older is well entrenched in gerontological thinking and is often used to "explain" age differences in performance. The evidence for this idea, however, is sparse and not consistent. It is the purpose of this project to explore the generality of the age-interference hypothesis for non-verbal memory and perception. It is important, both for theoretical and applied reasons, to identify those conditions which are especially interfering for the old.

Furthermore, correlative studies of interference behavior and physiological variables, such as the alpha period of the EEG, should improve our understanding of the processes underlying the behavior.

Proposed Course of Project: Declines in performance on memory-for-designs will be related to health and physiological variables (such as blood pressure and EEG). Data collection will be initiated for the study of the psychological refractory period.

Keyword Descriptors:
Aging, susceptibility to interference, perceptual retention, non-verbal memory, psychological refractory period.

Honors and Awards: None

Publications: None

Project No. Z01 AG 00067-08 LBS
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Learned Modification of Visceral Function in Man

Previous Serial Number: HD-LBS-8

Principal Investigator: Bernard T. Engel, Ph.D. (50%)

Other Investigators: Stanley A. Rubin, M.D. (90%)
Marvin M. Schuster, M.D. (5%)

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	2.75
Professional:	1.45
Other:	1.30

Project Description:

Objectives:

A. Learned control of blood pressure: 1) To develop a complete physiologic characterization of the cardiovascular response to learned blood pressure control; 2) To assess the efficacy of outpatient training of hypertensives learning blood pressure control; 3) To compare the strain gage plethysmography with the water-filled plethysmograph.

B. Learned control of rectal function: 1) To determine whether patients with irritable bowel syndrome (IBS) can be trained to control recto-motor responses; 2) To determine the range of clinical application of anal sphincter training among patients with chronic severe fecal incontinence.

Methods Employed:

A. Learned control of blood pressure. The operant conditioning paradigm which employs the automatic cuff-Korotkoff sound detector is utilized for teaching Ss to alter systolic blood pressure. Patients with mild to moderate hypertension are culled from the Baltimore City Hospital clinics, from the Gerontology Research Center longitudinal study and from physician referrals. Raising and lowering systolic blood pressure is learned while participating in laboratory training as an outpatient (low intensity learning schedule) or as an inpatient (high intensity learning schedule). This is followed by a brief inpatient study of cardiovascular parameters to define the physiological mechanisms mediating learned blood pressure control. Strain gage

plethysmography of arm or leg will yield information on forearm blood flow and venous capacitance; cardio-ultrasound will measure left ventricular transverse diameter. Intra-arterial pressure measurements also will be made. Additional parameters which can be derived from these measurements include cardiac output, systemic vascular resistance, limb vascular resistance and mean arterial pressure. Blood pressure raising against trimetaphan, and lowering against atropine and isoproterenol will be studied following the acquisition of high level performance. In collaboration with the Nephrology Division of the Johns Hopkins Hospital, venous renin and angiotensin and arterial angiotensin will be assayed.

B. Limb plethysmography. Small, lightweight mercury-in-silastic rubber gages encircle the limb at mid-forearm or calf. A narrow pressure cuff at the wrist or ankle is inflated to suprasystolic level to occlude distal blood flow, and a proximal cuff is inflated to restrict venous outflow. Limb circumference changes are transduced into electrical signals which are amplified and recorded. Rapid inflation of the collecting (proximal) cuff to 30 mm Hg results in unimpeded arterial inflow. Limb girth increases proportional to limb blood flow. Collection lasts for 5 seconds followed by 5 seconds of relaxation. Limb blood flow measurements for 5 second epochs over a two minute period are obtained by alternating series of collection periods.

C. Learned control of recto-motor function. Rectal activity is assessed by means of pressure recordings from a multiple balloon system. One balloon is placed high in the rectum and is used to stimulate the rectum. A second balloon is placed lower in the rectum and is used to record recto-motor responses. A third balloon is placed at the internal anal sphincter and also is used to record motor activity. The high rectal balloon is progressively more inflated so that the rectum is increasingly distended. Discrete inflation/deflation pulses are superimposed on a baseline level of rectal distension. Thus, two kinds of stimulation, sustained distension and discrete distension, are used. In the normal, sustained distension has no effect on rectal motility after a single response to the onset of inflation. Discrete distension produces a single wave of relaxation. In patients with IBS, sustained distension often results in periods of spontaneous rectal and internal sphincteric relaxation and contraction; discrete distension usually produces multiple responses in the rectum and at the internal sphincter.

Major Findings:

A. Learned control of blood pressure. Studies have just begun and no results are available.

B. Limb plethysmography. Preliminary data indicate that blood flow measurements made over a 2 minute period following wrist occlusion follow a substantially different pattern than has been reported for the water-filled plethysmograph. The curve of blood flow is considerably flattened over time; this is in contrast to other data showing a biphasic flow pattern within the first minute of wrist occlusion with subsequent damping.

C Learned control of recto-motor function. Progress has been limited. We have seen only two patients with IBS: one of whom also was incontinent, and one patient with fecal incontinence -- n.b., it should be clear that the gastrointestinal service continues to see and to treat incontinent patients

using operant procedures. This is now routine therapy. For the purposes of this project the only patients being considered are those who would permit us to assess the limits of application such as patients with histories of myelomenigocele or paraplegic patients. With respect to the two patients with irritable bowel syndrome, both showed evidence of recto-motor control in the laboratory; however, only the patient who also was incontinent reported significant clinical improvement.

Significance: This project is designed to increase our understanding of learned control of autonomic responses and the application of this knowledge to the control of various illnesses. These data add further credence to the conclusion that responses mediated by the autonomic nervous system can be brought under voluntary control, and they show that such control holds out the possibility of clinically useful applications.

Proposed future course: The projects described above will be continued.

Keyword Descriptors: High blood pressure, Operant conditioning, Biofeedback, Plethysmography, Irritable bowel, Rectal motility.

Honors and Awards:

Dr. Engel conducted medical grand rounds, University of Chicago School of Medicine (October, 1974).

Dr. Engel led the psychiatry staff conference, Johns Hopkins University School of Medicine (November, 1974).

Dr. Engel presented to the Johns Hopkins Medical and Surgical Association (February, 1975).

Dr. Engel gave an invited address at the 20th Annual Conference, V.A. studies, Mental Health and Behavioral Sciences (April, 1975).

Dr. Engel participated (by invitation) in a symposium of the American Psychiatric Association (May, 1975).

Dr. Engel participated in a course in continuing education for internists in Boston (June 1975).

Publications:

Engel, B. T. Clinical applications of "biofeedback" in the treatment of fecal incontinence and in the control of some cardiac arrhythmias. Tribuna Medica (Madrid). in press.

Kristt, D. A., and Engel, B. T. Learned control of blood pressure in patients with high blood pressure. Circulation, 1975, 51, 370-378.

Roessler, R. R. and Engel, B. T. The current status of the concepts of physiological response specificity and activation. Psychiatry in Medicine, in press.

Weiss, T. and Engel, B. T. Evaluation of an intra-cardiac limit of learned heart rate control. Psychophysiology, in press.

Project No. Z01 AG 00068-13 LBS
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Learning and Problem Solving Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Operant performance, memory and aging

Previous Serial Number: HD-LBS-9

Principal Investigator: Charles L. Goodrick, Ph.D. (10%)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total	.20
Professional:	.10
Others:	.10

Project Description:

Objectives: The general objectives of one phase of this project are: (1) to analyze complex maze learning of young and aged animals' and (2) to determine variables which may act to enhance or retard maze learning ability. The objectives of the other phase of this project are: (1) to study age differences in motor performance during operant responding; and (2) to develop operant techniques which improve the retention of learned responses in aged animals.

Methods Employed: Operant conditioning performance and retention studies have used 2-bar test boxes in which hungry animals are trained to press one bar to obtain a food reward while the alternate bar remains neutral. By increasing the complexity of the task (using two bars rather than one), it is possible to make a finer analysis of performance and the retention process. We are studying performance and retention as a function of reward schedule, and we are particularly interested in the partial reinforcement effect. The retention of partially rewarded responses is vastly greater than responses continuously rewarded; and analysis of this phenomenon will provide information regarding the general retention process.

A complex 14-unit multiple-T maze also is utilized. This maze has been shown to be a highly reliable test of learning, and it has been used in many major studies of aging. Additional mazes of 6-units are being developed to study mastery of consecutive problems by young and aged rats. These mazes will be used to analyze aging effects in short-term and long-term memory and to determine aging effects in interference, both proactive and retroactive.

Major Findings: Data are currently being analyzed which will be reported in detail in next year's annual report.

Significance to Bio-Medical Research and the Program of the Institute: Learning and/or memory deficits represent a major problem among the aged human population. Major behavioral techniques to reduce performance deficits obtained for aged animals have been studied in our laboratory. This project may facilitate research with man by identifying optimal conditions for learning and for retention of learned responses.

Proposed Course of Project: Further studies are in progress to determine the nature of the partial reinforcement effect in relation to (a) time contingent vs. response contingent partial reinforcement, and (b) massed vs. distributed extinction trials. Other studies will examine age differences in operant performance as a function of response effortfulness. Maze studies will concentrate upon the effects of central nervous system stimulants on behavioral rigidity within the maze for old rats. We will also initiate preliminary studies of perceptual learning in humans to determine the generality of the massed practice effects found for aged rats.

Keyword Descriptors: Learning, memory, age, rat.

Honors and Awards:
Dr. Goodrick was invited to participate in a symposium at the annual meeting of the Maryland Psychological Association, April, 1975. His presentation was entitled "Experimental studies of cognitive performance (complex maze learning) utilizing young and aged rats."

Dr. Goodrick presented a paper entitled "Use of forced correct responses in learning of a complex maze problem for mature-young and aged rats" at the annual meeting of the American Psychological Association, September, 1974.

Publications:
Goodrick, C. Primary and secondary motivational properties of a light stimulus. Psychological Reports, 1974, 34, 799-809.

Goodrick, C. Behavioral Rigidity as a mechanism for facilitation of problem solving for aged rats. Journal of Gerontology, 1975, 30, 181-184.

Project No. Z01 AG 00069-10 LBS
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Learning and Problem Solving Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Exercise, general activity level and aging

Previous Serial Number: HD-LBS-10

Principal Investigator: Charles L. Goodrick, Ph.D. (40%)

Other Investigator: John Holmblad (15%)

Cooperating Units: Baltimore City Hospitals

Man Years:

Total	.95
Professional:	.55
Others:	.40

Project Description:

Objectives: The general objectives are: (1) to determine methods for increasing vigorous physical activity of lower animals during late stages in the life span (2) to examine behavioral and longevity differences among animals which differ in physical activity level, and (3) to determine the physiological mechanisms underlying differences in activity.

Methods Employed: Wistar rats or various strains of mice are placed in standard activity wheels and allowed access to free voluntary exercise (VWE). Hungry animals also may be rewarded with food for running. Other studies utilize inbred, hybrid, and mutant mice or species which differ in activity level due to different genetic constitutions. (See Project HD-LBS 1 Behavioral genetics and aging).

Major Findings:

A. Last year we reported that a major investigation was in progress using rats to determine the effects of socialization during wheel exercise upon body weight, metabolic rate, and longevity. for rats paired in the activity wheels, it was found that body weights were much lower than for rats singly caged in activity wheels. In addition, mean wheel activity per rat was higher for paired rats than singly caged rats. The body weight of singly caged rats in activity wheels became very high at 7-8 months of age, similar to body weights of rats kept in cages without access to wheels, while wheel activity was reduced to virtually zero. As a result of these findings, the singly caged rats were discontinued, and we have concentrated on paired rats maintained in cages with attached activity wheels or in cages without access to activity wheels.

There are currently two sets of rats which are being maintained. The 40 males and 40 females allowed access to activity wheels were started under these conditions when 45 days old. Of the animals which are now 22 months old (Group 1, N = 24) and in activity wheels 22 are in good condition, with one in poor condition (very debilitated or with large tumors) and one death. The controls (not allowed access to activity wheels) which are 22 months old have 30 of 60 animals remaining in good condition, with 8 in poor condition and 22 deaths. For the groups in the wheels which are now 16 months old (Group 2, 28 male and 28 female), there has been one death. Although these results are incomplete, the evidence is strong that voluntary wheel exercise is highly beneficial to the health of these animals.

The mean body weight of Wistar rats, both male and female, allowed voluntary wheel exercise was significantly less that that of caged rats with no access to activity wheels. These weight differences increased with age, reaching a maximum at 12 mo. for males (163.2 gm.) and a maximum at 15 mo. and 18 mo. for females (97.7 and 99.0 gm). The metabolic rate of female rats was found to be significantly greater than for male rats. For control rats, metabolic rate decreased with increasing age from 3 to 21 mo. of age, while metabolic rate remained constant for wheel activity groups tested at these ages. As a result, the mean metabolic rate of rats allowed VWE was significantly higher than the metabolic rate of controls tested at 12 to 21 mo. of age.

Wheel activity over the life span will be discussed in detail in the report next year when the data are complete.

B. Data for a doctoral dissertation has been collected by Ms. Teena Wax, working within our laboratory. This thesis is concerned with the effect of age (young or senescent), group (A/J, C57BL/6J, and F_1 hybrid), and lighting condition (high or low illumination) upon circadian periodicity (wheel, bar press, light) in a free choice situation where the animal controls light onset and offset. The thesis data analysis and writing is nearing completion and will be presented in detail in next year's report.

C. It has been suggested that animals dissipate food energy in three primary ways: as mechanical work, as heat, and as energy stored in the form of protein, fat, or carbohydrate. At the present time little is known of the genetic and behavioral mechanisms which regulate the rate of these energy exchanges and which determine whether food will be stored or transformed to work or heat. To obtain preliminary information with regard to this problem area, a study was initiated which measured food intake, voluntary wheel exercies (mechanical work), metabolic rate (heat), and body weight (energy stored) for groups of mice known to have different maximal body weights.

The relations of voluntary wheel exercise, food intake, water intake, metabolic rate, and body weight were determined for mutant mice (bg, c^J, A^y, and ob) with the C57BL/6J genetic background and also for littermate controls. Mutant groups high in body weight (A^y and ob) had lower metabolic rates, were less active, and ate more food than controls. The nonobese mutants (c^J, and bg) had higher metabolic rats than controls, but different behavioral mechanisms for control of body weight during voluntary wheel exercise.

Voluntary wheel activity for the albino (c^J) mice was similar to that of controls, but food intake increased proportionally more for albinos than controls. Food intake was similar for beige (bg) mutants and controls, but the beige group engaged in less voluntary wheel activity than controls.

As the control groups increased level of wheel running activity over sessions with wheel access, food and water intake also increased, and body weight remained constant. Long term studies during which wheel activity, food and water intake, body weights, and metabolic rate are studied will determine changes which occur with increasing age.

Significance to Bio-Medical Research and the Program of the Institute: One of the consistent findings of gerontological research is the decline in general activity level of old animals compared with young animals. It is important to determine whether quantity of activity (e.g., wheel activity) and/or quality of activity (e.g., increased exploration behavior or greater response variability) may be increased experimentally for old and senescent animals. It is also important to examine the role of heredity with respect to voluntary exercise throughout the entire lifespan, and the effect of exercise upon behavioral decrements associated with advanced old age. The knowledge and utilization of factors which change base activity levels of aged animals may result in more productive later years for aged humans.

Proposed course of the project: The studies of rat wheel exercise will continue to determine the effects of voluntary exercise upon longevity for paired rats, and to determine the amount of voluntary exercise during advanced old age.

Studies of wheel exercise periodicity of young and aged mice will be continued. Periodicity patterns of mice will be examined throughout old age. Additional studies will determine the level of voluntary activity for young and aged mice which are allowed voluntary control of lighting conditions within the home environment.

Keyword Descriptors: Exercise, activity, age, rat, mouse.

Honors and Awards: None

Publications:

Goodrick, C. Exploration behavior of immature albino rats. Developmental Psychology, 1974, 10, 438-441.

Goodrick, C. Adaptation to novel environments by the rat: Effects of age, stimulus intensity, group testing, and temperature. Developmental Psychobiology, In Press.

Wax, T. Runwheel activity patterns of mature-young and senescent mice: The effect of constant lighting conditions. Journal of Gerontology, 1975, 30, 22-27.

Wax, T., & Goodrick, C. Voluntary exposure to light by young and aged albino and pigmented inbred mice as a function of light intensity. Developmental Psychobiology, In Press.